Potential for Biodegradation of Sub-Littoral Residual Oil by Naturally Occurring Microorganisms following the Lake Wabamun Train Derailment

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to

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Executive Summary

Laboratory tests were conducted under two incubation conditions to estimate the hydrocarbon-degrading ability of microbes naturally present in oil-contaminated Lake Wabamun sediment. The tests incubated spilled Bunker C oil recovered from the lake and a reference oil, Alberta Sweet Mixed Blend (ASMB), at 22°C for 4 weeks or at 4°C for 8 weeks. Nutrients and aeration were provided to ensure suitable conditions for aerobic hydrocarbon degradation. Residual oil was recovered and analyzed quantitatively by gas chromatography (GC) for several classes of analytes, including Total Petroleum Hydrocarbons (TPH), Total Saturated Hydrocarbons (TSH) and Total Aromatic Hydrocarbons (TAH).

The lake sediment contains an active microbial community as determined by viable plate counts, and a substantial number of hydrocarbon-degrading microbes as estimated by the Most Probable Number (MPN) technique.

Lake Wabamun sediment microbes were capable of degrading a significant proportion of ASMB reference oil at both incubation temperatures. Up to 63% of the GC-detectable TPH was biodegraded. This included complete removal of *n*-alkanes and nearly complete (97%) removal of total Polycyclic Aromatic Hydrocarbons (PAH) at the warmer temperature. The results prove the existence of a significant hydrocarbon-degrading community of sediment microbes with broad substrate range, active at both 4°C and 22°C but showing more biodegradation at the higher temperature.

Biodegradation of Bunker C oil was less significant than ASMB, with much smaller masses of analytes being degraded under both incubation conditions. For example, only 126 mg of Bunker C TPH were degraded compared with 263 mg in parallel ASMB cultures. Under the best incubation conditions, biodegradation accounted for loss of only 12% by weight of the Bunker C oil added to the cultures. This reflects the small proportion of low-molecular weight hydrocarbons considered to be biodegradable and the high proportion of components that either are not readily biodegradable or are not GC-detectable in the spilled Bunker C oil.

In summary, competent hydrocarbon-degrading microbes exist in Lake Wabamun sediment. However, the mass of Bunker C oil that they are likely to be able to degrade is small due to the recalcitrant nature of the oil. This is likely exacerbated by the formation of "tar balls" which limit microbial access to the biodegradable components of the spilled oil.

Overall, the prognosis for extensive natural bioremediation over the short term, say 5 – 10 years, is poor, and these laboratory tests indicate that a large proportion of the spilled oil will persist even under optimum conditions for biodegradation.

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1. Introduction

On Wednesday August 3rd, 2005, 46 Canadian National Railway Company (CN) rail cars derailed approximately 65 km west of Edmonton, Alberta. Approximately 712,000 L of Bunker C heavy fuel oil and 88,000 L of Imperial Pole Treating Oil (PTO) (a relatively light hydrocarbon product containing polycyclic aromatic hydrocarbons) were released from the ruptured cars. The oils saturated the adjacent ground, flowed on the surface, and entered the lake waters on the north shore (approx. 53^0 34.045 N and 114^0 35.201 W) within a few hours of the derailment. A variety of scientific support surveys or activities were initiated by Alberta Environment and Environment Canada (Sergy and Zrum pers.com; Zrum et al., 2006) including:

- Monitoring the effects of the spilled substance on the water and the aquatic environment
- Systematic surveys and documentation of the affected shoreline
- Comprehensive analysis of the oil samples collected from the site post-spill, including full "fingerprinting" analysis and detailed physical and chemical analysis of "tar balls"
- Monitoring of water and sediment quality, and aquatic biota in the main body of the lake post-spill, including collection of benthic invertebrates, phytoplankton, zooplankton and fish samples
- Investigation of littoral areas of concern (migratory bird habitat, evidence of submerged and/or sunken oil in Fall 2005)
- Potential ability of Lake Wabamun sediment microorganisms to biodegrade spilled Bunker C oil

This report describes results from the last activity listed, and represents collaboration between Alberta Environment (AENV; partial funding and provision of samples), Environment Canada (EC; quantitative analysis as an in-kind contribution and provision of samples) and the University of Alberta (UA; microbiological tests and incubation).

Interpretation of the test results is supported by previous extensive collaborative work among EC Emergencies Science and Technology Division Environmental Technology Centre (EC-ETC) and UA, which included controlled tests to determine the biodegradability of Bunker C oil (Blenkinsopp et al. 1996; Wang et al. 1998). In that prior work, a sample of Bunker C provided by EC-ETC was incubated with defined oil-degrading microbial consortia (Wang et al. 1998; Foght et al. 1998). Analysis of the residual oil by EC-ETC using quantitative gas chromatography with a flame ionization detector (GC-FID) and GC with a mass selective detector (GC-mass spectrometry; GC-MS) showed that only a small proportion of the Bunker C oil was biodegradable by the consortium. This is because, generally speaking, increasing the molecular weight and complexity of hydrocarbons decreases their susceptibility to biodegradation, and "heavy" oils with a high proportion of such components tend to be poorly degradable.

Therefore, we assumed that the Bunker C oil spilled into Lake Wabamun would be similarly recalcitrant to biodegradation. However, the maximum amount of

biodegradation that could be achieved (the "biodegradation potential" of Bunker C) could not be predicted with confidence because the oil-degrading abilities of microbes naturally present in Lake Wabamun had not previously been determined. In the tests described below, Lake Wabamun sediment containing natural microbes was incubated in laboratory tests under ideal conditions to determine whether these microbes could degrade components of Bunker C oil that are detectable by GC. With suitable caution, the results can be extrapolated to biodegradation potential *in situ*.

2. Objectives

The project had two objectives:

- To determine the presence and ability of natural Lake Wabamun sediment microorganisms to biodegrade hydrocarbons under ideal laboratory conditions
- To estimate, under laboratory conditions, the biodegradation potential of residual Bunker C oil collected from the sub-littoral zone of Lake Wabamun

Both objectives include the understanding that less-than-ideal *in situ* conditions will likely result in lower levels of biodegradation over an equivalent time span than the optimized laboratory conditions employed in the tests.

To achieve these objectives, laboratory tests were performed by incubating natural microbes from Lake Wabamun with spilled oil recovered from the lake at two incubation temperatures. The residual oil was analyzed and compared to appropriate controls, including a reference oil, to estimate the mass of spilled Bunker C oil that is susceptible to biodegradation under ideal laboratory conditions.

3. Rationale

The trial was conducted according to the experimental matrix in Table 1, with Bunker C tests conducted in triplicate and sterile controls in duplicate. Single flasks of positive control cultures were incubated with Alberta Sweet Mixed Blend (ASMB), a reference oil previously tested by Environment Canada and known to be biodegradable (Wang et al. 1998).

Two sources of inoculum were used for the tests: fresh, oil-impacted sediment collected from Lake Wabamun, and a laboratory-incubated "enrichment culture" derived from Lake Wabamun sediment, described below. Lake sediment was used as a source of microorganisms rather than water column samples for several reasons: (a) there is a much higher density of cells in sediment than in water and therefore a greater likelihood of oil-degrading microbes being present; (b) observations indicated that a substantial amount of oil had sunk to the bottom and was residing on or in the bottom substrate (Sergy and Zrum, pers.com); (c) immobilization of spilled oil by sorption to suspended fine particulates will also tend to deposit oil into the sediment for slow release to the surface or biodegradation; and (d) oil impacting the sediment will tend to persist there due to microaerobic or anaerobic conditions that slow oil biodegradation. That is, the long-term

impact of the oil is greater on the lake sediment than on the water column, even though, at least initially, it is more apparent and more accessible to physical recovery and abiotic losses (weathering) on the water surface. In addition to the fresh sediment inoculum, an enrichment culture was prepared by exposing sediment to spilled oil over three successive monthly transfers in the laboratory (described in detail in Section 4.1). This was designed to increase the proportion of microbes capable of degrading Bunker C by natural selection, and to ensure that oil-degrading microbes were available for the tests. In previous EC – UA tests for biodegradation potential, we used a defined consortium of microbes (Foght et al. 1998) as inoculum. This was not desirable for the current tests, because it is the natural microbial community that must deal with the spilled oil *in situ*.

Table 3. 1 Experimental matrix to determine the potential for Lake Wabamun sediment microorganisms to degrade spilled, recovered Bunker C oil or ASMB reference oil under ideal laboratory conditions at two temperatures.

Inoculum	**Oil**[a]	# replicate flasks to be analyzed after incubation at:	
		22°C for 4 weeks	**4°C for 8 weeks**
Fresh sediment[b]	Bunker C	3	3
	ASMB	1	1
Enriched sediment[c]	Bunker C	3	3
	ASMB	1	1
Sterile controls[d]	Bunker C	2	2
	ASMB	1	1

a, Bunker C was recovered from Lake Wabamun after the spill and represents partially weathered oil; ASMB is Alberta Sweet Mixed Blend oil, a reference crude oil known to be biodegradable (positive control)

b, Slurry of Lake Wabamun sediment and water, collected from a contaminated site

c, Lake Wabamun microbes enriched for hydrocarbon-degrading microbes by repeated laboratory transfer with Bunker C oil as sole carbon source (Section 4.1)

d, Heat-killed Lake Wabamun sediment, to account for sorptive and weathering losses during incubation

Two oils were used in the trials: Bunker C and Alberta Sweet Mixed Blend (ASMB). The Bunker C oil residue was collected from the sub-littoral zone of Lake Wabamun about 11 weeks after the spill at cessation of the 2005 cleanup operations in October. The sunken or submerged oil now being found in the lake would remain over the winter and therefore its fate, including biodegradability, was of interest[1]. By this point in time, the oil had been altered by environmental processes to various forms and weathering states. The form selected for the testing was one which was being commonly observed, was of environmental concern because of its relative freshness and stickiness, and appeared to be a form most likely to still contain low molecular weight material amenable to biodegradation. Most other forms appeared more weathered.

[1] This form was observed during winter sampling and was still prevalent in spring 2006. Detection and recovery of submerged oil particularly in reed beds became the prime thrust of the 2006 cleanup operations (Sergy pers.com)

Of potential relevance with respect to fate, is that the Bunker C was being transported at high temperature and was spilled and entered the lake when hot (G. Sergy, personal communication). Biodegradable volatile components could have been lost by volatilization at the beginning of the spill, leaving behind the higher molecular weight, recalcitrant components for bioremediation. Subsequently, the weathered oil might have lost some additional low molecular weight components through volatilization and dissolution processes in the lake. Using fresh Bunker C oil in the tests would likely give a false impression of biodegradation potential compared with this weathered oil, depleted in biodegradable compounds and would be of less relevance to projections of in-situ fate.

The second oil selected was ASMB, an oil shown to be biodegradable in previous tests (Foght et al. 1998). This oil was included as a positive control to demonstrate that hydrocarbon-degrading microorganisms were present in the inocula and active under the test incubation conditions. Sterile controls containing heat-killed sediment suspended in sterile medium, parallel to the test cultures, were included for both oils to account for non-biological losses due to sorption and volatilization.

Two incubation temperatures were chosen: 22°C, representing summertime conditions and 4°C, representing wintertime conditions. Because microbial activity is slower at low temperatures, the incubation time at 4°C was extended to 8 weeks, whereas at 22°C incubation continued for 4 weeks. These time periods previously have been determined to be suitable for laboratory incubations at these temperatures (Blenkinsopp et al. 1996; Foght et al. 1998).

Tests were conducted under "ideal conditions", defined here as liquid Freshwater Medium amended with nutrients (ammonium nitrate and potassium phosphate at pH 7), and incubation with gyrotory shaking to provide aeration. Optimum hydrocarbon degradation generally requires the presence of N and P and aerobic biodegradation requires O_2. No chemical agents were added to the oil to disperse it to increase surface area for biodegradation, but the gyrotory shaking did help to break the oil into small droplets and balls, increasing available surface area. A small volume ratio of oil:culture medium was selected, based on previous biodegradation studies (Foght et al. 2004).

4. Methods

Additional methodological details such as media recipes are presented in Appendix 1.

4.1 Establishing enrichment cultures

Five enrichment cultures were established with different combinations of inocula and oils (Table 4.1), to ensure a selection of competent microbes for the tests. Oil-impacted sediment plus overlying water was collected from Lake Wabamun in a heavy-gauge polyethylene bag on October 20, 2005 from site SA-07 (Freeman Reed Bed) During collection, the sediment bed emitted particulate oil when disturbed, thus confirming the in-situ presence of oil-impacted sediment (see Figure 1). The sediment was delivered to the UA laboratory the following day and stored at 4°C in the dark. The sediment, with overlying water, was black and sandy with occasional bits of vegetation (grass or reeds)

and an odour of oil and slight H_2S. It released slight surface sheen when added to liquid medium.

Figure 4. 1 Resurfacing oil in Freeman reed bed sediments, Lake Wabamun

On October 20, 2005, EC and AENV personnel collected the Bunker C test oil from Lake Wabamun at 5 – 50 cm water depth, 1-5 m offshore at site SG-01. The sample consisted of a composite of small oil balls, 2-3 cm in diameter, mostly spherical in shape and having slightly negative buoyancy. They were soft, with a thin exterior skin containing a relatively fresh oil interior (fluid and sticky). These oil balls appeared to be one of the least weathered forms of submerged oil (Sergy pers.com). An example of this oil can be seen in Figure 2. The oil was placed in a wide-mouth glass container with a screw cap, and delivered to the UA laboratory the next day, where it was stored at 4°C in the dark. The oil was highly viscous, with the consistency of a thick tar, but it did spread into a discontinuous slick on the surface of liquid medium if left for a few hours at room temperature.

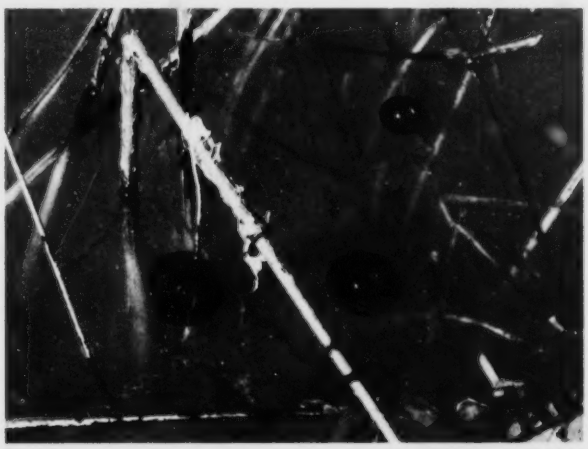

Figure 4. 2 Floating tar balls, section G-01, Lake Wabamun

On October 24, 2005, the sediment slurry was mixed by hand and 40 mL were inoculated into each of four 500-mL Erlenmeyer flasks containing 200 mL of sterile Bushnell-Haas medium (Appendix 1). A fifth flask of medium was inoculated with an established laboratory culture ("SLvsPB") known to comprise a mixed community of competent oil-degrading microbes. To three of the flasks, approximately 400 mg of recovered Bunker C oil was added by dropping lumps of oil into the medium as sole carbon source for microbial growth. To two flasks, 1 mL of ASMB oil was added to provide readily degradable hydrocarbons for the microbes, should the Bunker C prove to be a poor enrichment substrate. The five flasks were incubated as shown in Table 4.1 and transferred three times at approximately monthly intervals by inoculating 10 mL of culture into 200 mL fresh sterile medium and adding fresh oil. This process has been shown previously to be effective at enriching hydrocarbon-degrading microbes from environmental samples.

Table 4. 1 Enrichment cultures inoculated on October 24, 2005.

Culture #	Oil	Inoculum	Incubation Temperature
1	Bunker C	Wabamun Sediment	22°C
2	Bunker C	Wabamun Sediment	10°C
3	ASMB	Wabamun Sediment	22°C
4	ASMB	Wabamun Sediment	10°C
5	Bunker C	SLvsPB culture	22°C

After three months of transfers, all cultures showed growth (turbidity) and had altered the appearance of the oils (small globules of oil, strings of oil suspended in brown medium), indicating that all enrichments contained hydrocarbon-degrading microbes. Culture #2 was selected from these enrichments because it was the best analogue for the *in situ* conditions in Lake Wabamun (i.e., sediment as inoculum, Bunker C oil as sole carbon source, and an incubation temperature intermediate between the two planned test temperatures).

4.2 Estimating viable numbers in sediment and enrichment culture inocula

The number of viable aerobic heterotrophs (microbes utilizing organic carbon in the presence of O_2) was determined for Enrichment Culture #2 and for the sediment slurry. Viable numbers were determined using the standard plate count method by diluting a known volume of slurry or culture using 10-fold serial dilutions in 10 mM potassium phosphate buffer (pH 7), then plating 0.1 mL of each dilution onto Plate Count Agar (Difco) in triplicate (Foght and Aislabie 2005). Plates were incubated at 22°C for 5 days, after which colonies were counted on appropriately diluted plates.

The number of hydrocarbon-degrading microbes was estimated using a standard 3-tube Most Probable Number (MPN) technique (Foght and Aislabie 2005). Test tubes containing 9 mL of Bushnell-Haas medium were inoculated in triplicate with 1-mL

volumes of serial 10-fold dilutions. Two drops of ASMB oil was added to each tube as sole carbon source, and the tubes were incubated at 22°C and scored at 1 week and 4 weeks, stationary, in the dark. A positive result is noted as reduction of the oil sheen or significant change in physical appearance of the oil compared with an uninoculated control tube, and also with increased turbidity of the liquid medium due to growth of microbes. Standard statistical tests (Eaton et al. 1995) were applied to estimate the most probable number of hydrocarbon-degraders present in the original inoculum.

4.3 Preparation of Test and Control cultures

Test and Positive Control flasks were prepared using 500-mL Erlenmeyer flasks containing 200 mL sterile Freshwater medium (Appendix 1). Sterile Control flasks received only 100 mL medium in preparation for addition of 100 mL of sterilized sediment slurry (see below).

The Bunker C oil recovered from Lake Wabamun contained fine sediments and some entrained water. To remove the fines and water and make the oil homogeneous, approximately 30 g of recovered oil was dissolved in 300 mL of methylene chloride (HPLC grade, Fisher). This solution was pre-filtered through a Whatman FH 0.22 μm pore size filter to remove the particulates, then filter-sterilized through a Millex FG 0.22 μm pore size filter (Millipore). Exactly 5.0 mL of solution, representing 0.392 g ± 0.004 (n = 5) of recovered Bunker C oil, was dispensed by volumetric pipette to each uninoculated Test or Control flask of sterile medium, and the flasks were shaken at 22°C for ~72 h to remove the methylene chloride solvent. Although the flasks were prepared as replicates, some differences were noted in the appearance of the oil on the surface of the flasks at this point, with oil in some flasks spread fairly evenly and others with discontinuous drops. The different-appearing flasks were distributed throughout the test matrix so that no single treatment received a higher complement of unusual flasks. ASMB oil was added gravimetrically using a tared sterile glass syringe, weighing before and after each addition of oil to provide approximately 400 mg of ASMB per flask, weighed to 1 mg. No solvent was required and therefore no pre-incubation step with shaking was performed.

Four millilitres of filter-sterilized Standard N,P Nutrient Solution (Appendix 1) was added to all flasks. The Test cultures were then inoculated with either 10 mL of Enrichment Culture #2 or with a slurry of fresh Lake Wabamun sediment as follows: the bulk sediment sample was mixed by hand to suspend sediment in the overlying water and 20 mL of homogenized suspension was added to each flask. The dry solids content of the suspension was determined by drying triplicate aliquots of 10 mL of slurry to constant weight at 105°C. The Sterile Control flasks with 100 mL of medium were "inoculated" with a slurry of 20 mL of sediment in 100 mL of Freshwater medium that had been heat-sterilized by autoclave for 30 min on each of two successive days.

All flasks were incubated with gyrotory shaking (150 rpm) in the dark at either 22°C for 4 weeks or 4°C for 8 weeks.

4.4 Extracting residual oil after incubation

After incubation, flask contents were acidified to pH ≤2 with 4 N H_2SO_4. Then 100 µL of a surrogate standard solution were added to the flasks to achieve final masses of 1.2296 mg squalane (Sigma), 0.4236 mg *o*-terphenyl (99%, Aldrich) and 0.0016 mg d_{10}-phenanthrene (97 atom%, Aldrich) per flask, used as extraction controls for GC analysis. Fifty millilitres of dichloromethane was added to the flasks and mixed by vigorous swirling. For all flasks containing sediment, the liquid was decanted into a separatory funnel and the sediment was removed by filtration through a Whatman No. 1 filter to prevent clogging of the separatory funnel. The sediment was mixed with anhydrous sodium sulfate and rinsed with methylene chloride into the separatory funnel. For flasks containing enrichment culture, standard liquid-liquid solvent extraction was performed. All cultures were extracted exhaustively with methylene chloride in a separatory funnel (at least 3 x 50 mL). The extract was dried by passage through anhydrous sodium sulfate, concentrated on a rotovap, and transferred to tared vials. Solvent was removed by passage of N_2 gas over the surface of the extracts or by air flow in a fume hood.

For cultures incubated at 22°C for 4 weeks, the mass of recovered oil was estimated by weighing the mass of oil in the tared vials after removal of the solvent (Appendix 2). These recoveries ranged from 80 – 145% for the Bunker C oil, and 47 – 68% for the ASMB oil (lower recovery presumably is due to volatilization losses during incubation of this lighter oil). However, we did not know whether the methylene chloride was completely removed from the Bunker C extracts, which would artificially increase the apparent recoveries. Also, EC-ETC observed fine particulates in the extracts (Z. Wang, pers. comm.), possibly from sediment fines or from the anhydrous sodium sulfate, which also would increase the apparent recovery of Bunker C oil. Therefore, we did not determine gravimetric recovery for the second batch of cultures incubated at 4°C for 8 weeks because the numbers were not reliable, nor were they necessary because the surrogate standards added before extraction were used to account for small variations in extraction efficiency and any residual solvent or the presence of fines in the extract. Mass losses from the flasks were therefore calculated based on the weight of oil added to the flasks. The important point is that the recoveries for tests and parallel sterile controls were similar, indicating that the extraction method was consistent for all sets of extractions and the results are comparable.

4.5 Quantitative analysis of residual oil

All extracts were shipped to EC-ETC for analysis using standard methods developed by EC-ETC (Wang et al. 1998; see Appendices 2 and 3 for details). GC-detectable components were quantified using GC-FID and GC-MS, analyzing for:

- Total Petroleum Hydrocarbons (TPH), comprising GC-detectable components of the residual oil, including GC-resolvable peaks and the unresolved complex mixture (UCM; "hump")
- Total Saturated Hydrocarbons (TSH), including straight-chain, branched-chain and cyclic alkanes
- *n*-Alkanes, comprising *n*-C_8 – *n*C_{41} plus the isoprenoids pristane and phytane, a sub-set of TSH

- Total Aromatic Hydrocarbons (TAH), comprising hydrocarbons and aromatic heterocycles such as dibenzothiophene
- Total Polycyclic Aromatic Hydrocarbons (PAH) and a subset comprising five homologous series of alkylated aromatic hydrocarbons and their parent compounds (5-PAH)
- Biomarkers including terpanes and steranes

5. Results

5.1 Viable numbers and hydrocarbon-degrading bacteria in the inocula

Plate count and MPN estimates of aerobic heterotrophs and hydrocarbon degraders, respectively, are shown in Table 5.1. A population of 10^6 colony forming units (CFU)/mL in the enrichment culture is a reasonable cell density considering that Bunker C oil was the sole carbon source provided in the enrichment culture (contrast with, say, *E. coli* grown in a rich organic medium that might achieve 10^9 CFU/mL). The 20-fold lower viable numbers (~10^5 CFU/mL) in the sediment inoculum reflects that fact that it was a slurry of lake sediment and water with a solids content of 0.20 ± 0.11 g dry weight /mL (n=3), giving 4.7 x 10^5 CFU/g dry weight. Note that the standard plate count results represent only those microbes able to grow aerobically; the lake sediment may also harbour a large population of anaerobic microbes that would not be enumerated using this method. However, because aerobic microbes typically exhibit more rapid attack on substrates than anaerobes, only the aerobes were enumerated.

Table 5. 1 Summary of viable aerobic microbes and hydrocarbon-degrading microbes in the two inocula used for biodegradation tests.

Inoculum	**Viable Numbers** [a] **(CFU/ml)**	**Hydrocarbon degraders** [b] **(MPN/mL)**	
	5 days	1 week	4 weeks
Enrichment Culture #2	1.9 x 10^6 (± 0.16)	1.5 x 10^4 (0.3 – 4.4)	2.3 x 10^6 (0.4 – 12)
Lake Wabamun sediment slurry	9.4 x 10^4 (± 4.5)	2.1 x 10^4 (0.4 – 4.7)	2.3 x 10^5 (0.4 – 12)

a, colony forming units (CFU) per mL of inoculum (enrichment culture or sediment slurry); mean ± 1 standard deviation (n = 3 plates per dilution).
b, most probable number (MPN) per mL of inoculum (enrichment culture or sediment slurry); MPN (95% confidence intervals).

The proportion of hydrocarbon degraders in both inocula was very high, and actually slightly exceeded the viable number estimates by the end of the 4-week incubation period. That is, both inocula were enriched in microbes capable of degrading the ASMB oil used as the indicator in the MPN test. A high proportion of hydrocarbon-degraders in

the enrichment culture was expected since oil was the sole carbon source provided and natural selection would favor oil degraders, but the high proportion in the sediment inoculum was unexpected. This may be in part because colonies on plates were counted after only 5 days (a typical incubation time), after which the plates became overgrown with large colonies and could no longer be counted accurately, whereas the MPN tubes were incubated for 4 weeks, perhaps allowing slow-growing bacteria additional time to grow and have an effect on the indicator oil. This explanation is supported by the MPN data increasing 10 to 100-fold with extended incubation. That is, the true number of viable aerobes is probably higher than measured here. Regardless, the substantial number of oil degraders in the sediment is encouraging, as it suggests that natural enrichment of the microbial population in the oil contaminated sediment has already occurred, either as a relatively rapid response to the present of spilled Bunker C oil, or through chronic exposure to low levels of hydrocarbons in the lake from industrial, recreational and/or natural sources. Pre-spill concentrations of 21 PAHs in Lake Wabamun sediments were detectable at levels higher than those in nearby lakes (Anderson, 2003) and may have provided conditions resulting in naturally elevated numbers of hydrocarbon-degrading bacteria.

5.2 Biodegradation at 22°C for 4 weeks

Residual oil was recovered from the cultures after incubation and analyzed using GC-FID and GC-MS. Masses of different analyte classes were normalized to the original mass of oil added to the samples, using the standards. The raw data for cultures incubated at 22°C for 4 weeks are presented in Appendix 2.

In the tables below, only the following analyte classes are discussed for the reasons given:

- TPH, because it represents the broadest range of GC-detectable oil components
- TSH and TAH because these major classes are considered to be the most biodegradable and therefore are most often analyzed for biodegradation
- Total *n*-alkanes and Total PAHs because they represent the most biodegradable subsets of the TSH and TAH, respectively. As well, the PAH class includes many of the US-EPA priority PAHs, which are contaminants of concern.

Data for other analytes are tabulated and presented graphically, and chromatograms are given for each residual oil fraction in Appendix 2. Data excerpted from Appendix 2 are summarized in Tables 5.2 and 5.3 and presented as the residual mass (i.e., analyte recovered after incubation and extraction) and the absolute mass lost compared to the sterile control (both normalized to 1 g of added oil), and as the % mass lost in comparison to the heat-killed sterile control (which accounts for non-biological losses such as volatilization and sorption to sediments).

Figure 5. 1 Replicate cultures after incubation with Bunker C oil at 22°C for 4 weeks. Some variation in appearance among replicates is evident in panel B. Turbidity in the Fresh Sediment and Sterile Control flasks is due to the presence of fine particulates in the sediment, whereas the Enrichment culture flasks were virtually sediment-free (due to repeated transfer of the inoculum into new medium), and the particulates represent residual oil and biomass.

Table 5. 2 Calculated losses from cultures incubated at 22°C for 4 weeks with Bunker C oil. "Biodegradation" is calculated as the difference between the analyte remaining in the test cultures and the sterile controls, expressed as an absolute mass and as a percent of the sterile control analyte.

Analyte Class	Residual mass, mg/g sample [a] Mean ± 1 stdev			Biodegradation, mg/g sample (% loss vs. sterile control)	
	Sterile Control (n=2)	Fresh Sediment (n=3)	Enrichment Culture (n=3)	Fresh sediment	Enrichment culture
TPH	**239** ± 9.9	**114** ± 24	**131** ± 13	**126** (53%)	**108** (45%)
TSH	**133** ± 5.7	**79.1** ± 16.1	**80.7** ± 6.4	**54** (41%)	**52** (39%)
TAH	**106** ± 4.2	**34.6** ± 13	**50.3** ± 8.4	**71** (67%)	**56** (53%)
Total *n*-alkanes	**8.19** ± 0.54	**0.33** ± 0.10	**0.13** ± 0.06	**7.9** (96%)	**8.1** (98%)
Total PAHs	**32.6** ± 2.0	**4.75** ± 3.4	**3.66** ± 1.9	**28** (85%)	**29** (89%)

a, Data excerpted from Appendix 2

Table 5. 3 Calculated losses from cultures incubated at 22°C for 4 weeks with ASMB reference oil. "Biodegradation" is calculated as the difference between the analyte remaining in the test cultures and the sterile controls, expressed as an absolute mass and as a percent of the sterile control analyte.

Analyte Class	Mean mass, mg/g sample [a] (n=1)			Biodegradation, mg/g sample (% loss vs. sterile control)	
	Sterile Control	Fresh Sediment	Enrichment Culture	Fresh sediment	Enrichment culture
TPH	415	152	196	**263** (63%)	**219** (53%)
TSH	274	109	114	**165** (60%)	**160** (58%)
TAH	141	43.3	81.6	**98** (69%)	**59** (42%)
Total *n*-alkanes	46.8	0	0	**47** (100%)	**47** (100%)
Total PAHs	10.7	0.31	0.42	**10.3** (97%)	**10.3** (96%)

a, Data excerpted from Appendix 2.

The data in Table 5.2 show good replication among triplicate test cultures and duplicate sterile control flasks for Bunker C (ASMB tests and controls were single flasks). For both oils, the losses from the fresh sediment and enrichment culture inocula were similar (an exception is the TAH value for the ASMB oil in Table 5.3, but this was based on a single culture rather than replicates). Up to 53% of the GC-detectable TPH in Bunker C was lost by biodegradation, and nearly all of the Total *n*-alkanes (96%) and Total PAHs (85 – 89%) were biodegraded. These analytes represent sub-sets of the TSH and TAH, respectively, which were depleted to a lesser extent (up to 41% and 53 – 67%, respectively). That is, biodegradation was similar in both the fresh sediment and the enrichment culture inoculum, indicating that it does not seem to be necessary to perform an artificial (laboratory) enrichment process for oil-degrading microbes in contaminated Lake Wabamun sediment; rather, the natural microbial community is already competent for oil biodegradation.

Significant mass losses and % losses of ASMB were detected in the test cultures (Table 5.3), indicating that the microbial populations in both inocula were competent for oil biodegradation. However, both the masses and the % losses of all but one of the analytes were less with Bunker C (Table 5.2) than with ASMB (Table 5.3). The exception is the mass loss of Total PAHs, which was greater from Bunker C (~29 mg) than from ASMB (~10 mg), although the % loss compared with the sterile control was similar (~85% vs ~96%).

It is clear from the Sterile Control data in Tables 5.2 and 5.3 that there is more GC-detectable material (TPH) in ASMB (415 mg) than in Bunker C (239 mg) after incubation at 22°C for 4 weeks. As well, the microbes were able to degrade more of that TPH, both in absolute terms (mg/g sample) and in relative terms (% loss compared to sterile control) in the ASMB than in the Bunker C. That is, although the microbes are

good at oil degradation, they do not perform as well with Bunker C because less of it is biodegradable. For example, the fresh sediment was able to degrade 165 mg of Total Saturated Hydrocarbons in ASMB (Table 5.3) but only 54 mg in Bunker C (Table 5.2), a 3-fold difference. Likewise, the enrichment culture degraded twice as much TPH present in ASMB (219 mg; Table 5.3) as in Bunker C (108 mg; Table 5.2). The difference is not as apparent when considering % degradation because there was initially less TPH in the Bunker C (only 239 mg, vs. 415 mg ASMB TPH). Therefore, the absolute mass (mg) losses must be considered in addition to the relative (%) losses. This distinction is discussed in more detail in Section 6 and Table 6.1.

5.3 Biodegradation at 4°C for 8 weeks

Replication of cultures with Bunker C was generally better at 4°C (Table 5.4) than at 22°C. Whereas ASMB experienced greater abiotic losses (e.g, volatilization) with 8 weeks incubation at 4°C than with 4 weeks incubation at 22°C (shown by higher residual analytes in the sterile controls at the higher temperature, Tables 5.3 and 5.5), the Bunker C did not show appreciable differences in abiotic losses at 22°C versus 4°C (Tables 5.2 and 5.4). That is, the residual mass of all analytes in the Sterile Controls were similar for Bunker C at both temperatures (e.g., 239 ± 9.9 mg TPH after 4 weeks at 22°C versus 234 ± 1.4 mg TPH after 8 weeks at 4°C). This suggests that little low molecular weight volatile material (usually considered to be more biodegradable) is present in the Bunker C used in the tests.

Table 5. 4 Calculated losses from cultures incubated at 4°C for 8 weeks with Bunker C oil. "Biodegradation" is calculated as the difference between the analyte remaining in the test cultures and the sterile controls, expressed as an absolute mass and as a percent of the sterile control analyte.

Analyte Class	Residual mass, mg/g sample [a] Mean ± 1 stdev			Biodegradation, mg/g sample (% loss vs. sterile control)	
	Sterile Control (n=2)	Fresh Sediment (n=3)	Enrichment Culture (n=3)	Fresh sediment	Enrichment culture
TPH	**234** ± 1.4	**172** ± 15.5	**161** ± 10.6	**62** (27%)	**73** (31%)
TSH	**133** ± 0.7	**119** ± 8.7	**108** ± 7.8	**14.0** (11%)	**25** (19%)
TAH	**102** ± 1.4	**52.8** ± 8.5	**53.3** ± 3.2	**49.2** (48%)	**48.7** (48%)
Total *n*-alkanes	**8.4** ± 0.13	**1.8** ± 1.4	**0.65** ± 0.8	**6.6** (79%)	**7.8** (93%)
Total PAHs	**30.4** ± 2.4	**9.8** ± 3.8	**11.7** ± 2.78	**20.6** (68%)	**18.7** (62%)

a, Data excerpted from Appendix 3.

Table 5. 5 Calculated losses from cultures incubated at 4°C for 8 weeks with ASMB reference oil. "Biodegradation" is calculated as the difference between the analyte remaining in the test cultures and the sterile controls, expressed as an absolute mass and as a percent of the sterile control analyte.

Analyte Class	Mean mass, mg/g sample [a] (n=1)			Biodegradation, mg/g sample (% loss vs. sterile control)	
	Sterile Control	Fresh Sediment	Enrichment Culture	Fresh sediment	Enrichment culture
TPH	315	133	250	**182** (58%)	**65** (21%)
TSH	230	100	175	**130** (57%)	**55** (24%)
TAH	85	33.0	75	**52** (61%)	**10** (12%)
Total *n*-alkanes	41.8	1.33	1.08	**40.5** (97%)	**40.7** (97%)
Total PAHs	7.75	0.51	2.52	**7.24** (93%)	**5.23** (67%)

a, Data excerpted from Appendix 3.

Biodegradation generally was greater at 22°C than at 4°C for both Bunker C and ASMB oils. For example, the fresh sediment removed 126 mg (53%) of Bunker C TPH at 22°C and only 62 mg (27%) at 4°C. This trend was also apparent with ASMB and was similar for all analytes in both oils, as would be expected considering that biological processes increase with temperature to an optimum value, which must be >4°C for the Lake Wabamun sediment microbes.

6. Interpretation

6.1 Microbial numbers in enrichment culture inoculum vs. fresh sediment

The aerobic viable plate count is a general indicator of the "health" of the microbial population, although it does not necessarily enumerate every microbe present. This number should exceed or equal the number of aerobic hydrocarbon degraders in the inocula, with the enrichment culture expected to have the greater proportion of degraders because of the selection process. Instead, both inocula had comparable viable numbers and high proportions of hydrocarbon degraders. This indicates that the contaminated Lake Wabamun sediment already harboured a microbial community capable of attacking the ASMB oil used in the MPN test. This competent community may have originated by natural selection in response to acute contamination by the spill event, or by enrichment due to chronic low-level hydrocarbon contamination in the sediment: as noted in Section 5.1, Lake Wabamun sediment contains higher levels of aromatic hydrocarbons than near-by lakes (Anderson, 2003), possibly accounting for the enrichment observed.

Although it would have been more relevant to use Bunker C oil as the substrate for MPN tests, it was not used because at the beginning of the project the degradability of the Bunker C and our ability to visually detect its biodegradation were unknown. The test is based on visual changes in the physical properties of the oil (e.g. emulsification, change in appearance from a surface sheen to submerged threads, etc.) and we did not know whether the changes to Bunker C would be sufficient for sensitive detection. Therefore, the MPN of hydrocarbon degraders is a generous estimate based on the more biodegradable ASMB oil rather than on the actual spilled oil. However, the high proportion of hydrocarbon degraders is still an indicator of the good biodegradative potential of the natural sediment microbial community and suggests that the presence of the hydrocarbon-degrading microbes is not a transient phenomenon.

6.2 Biodegradation achieved in test and control cultures

The biodegradation tests were conducted under "ideal" laboratory conditions, as defined in previous studies using a consortium of laboratory microbes (Foght et al. 1998). We have assumed here that the same conditions would be suitable for the natural Lake Wabamun microbes, and based on work with N. Saskatchewan River water samples and soils (Foght and Semple, 2003). The suite of conditions (temperature and duration of incubation, volume of medium in flasks, agitation rate, volume ratio of oil:medium, concentration of nutrient amendments, etc.) have not been specifically optimized for Lake Wabamun microbes or designed to mimic *in situ* conditions, but the two temperatures and lengths of incubation are reasonable for estimating biodegradation potential. The analyte classes monitored in this trial are those routinely used by EC-ETC for biodegradation estimates (Wang et al. 1998) and exceed in both scope and accuracy the analysis of saturates and aromatics typically reported in the literature. The method is sensitive, quantitative and is supported by excellent replication (Appendices 2 and 3). However, it is important to recognize that these methods are limited to monitoring only the GC-detectable components. For oils such as ASMB this is not a significant problem because a large proportion of the oil mass is GC-detectable, but for heavy oils such as Bunker C, a smaller proportion of the oil can be monitored by this method. That is, Bunker C oil has a lower mass of GC-detectable material, as seen by the TPH value of only 239 ± 1.4 mg/g sample for the sterile controls (Table 5.2) versus 415 mg TPH/g sample for ASMB (Table 5.5) after 4 weeks incubation at 22°C. That is, only 24% of the total mass of Bunker C oil in the sterile control can be accounted for by GC analysis, whereas ~42% of the ASMB was detected. Within the GC-detectable material, the GC-resolved peaks are an even smaller sub-set of the TPH; the unresolved complex mixture (the "hump") can represent a large proportion of the TPH of oils like Bunker C (see chromatograms in Appendices 2 and 3).

The components that cannot be monitored by GC include the asphaltenes and resins, poorly defined classes of compounds comprising a heterogeneous mixture of hundreds or thousands of compounds believed to be poorly degradable. To overcome the problem of GC detection, gravimetric analysis has been used in other studies to measure the total mass of oil lost to biodegradation regardless of analyte class. However, gravimetric analysis can be inaccurate and variable, and it does not indicate whether compounds of particular interest have been degraded. Therefore, despite the inherent shortcomings of GC analysis as applied to heavy oils, it is still the best method available, but it is

important when interpreting the data to remember the methodological limitations.

Good replication was noted among triplicate tests and duplicate sterile control results. In biological systems, particularly those that have complex communities and/or are incubated for a long period of time, variation among replicates is expected. If the standard deviation falls within 10% of the mean, replication can be considered good. In this case it indicates that the extraction and analysis of residual oil were well-performed.

Results from parallel fresh sediment and enrichment culture inocula were similar for each oil under each incubation condition. This indicates that the lake sediment collected from the contaminated site already harboured a natural microbial population capable of degrading Bunker C oil, without having to impose an artificial selection process to enhance the hydrocarbon degraders. In addition, significant biodegradation was observed at both 22°C and at 4°C, showing that the sediment communities are able to degrade Bunker C under temperatures simulating summertime and wintertime. We do not know the rate of degradation of various components because only single timepoint measurements were taken at each temperature, but it is likely that degradation is slower at the lower temperature, based on previous observations (Blenkinsopp et al. 1996). The mass losses in test flasks were greater at 22°C than at 4°C for both oils, despite the longer incubation time at the lower temperature, suggesting that the consensus optimum temperature for biodegradation is above 4°C.

Because abiotic processes such as volatility and dissolution represent minor losses from Bunker C, biodegradation becomes even more important to clean-up. Therefore, to emphasize the biodegradation potential of Bunker C, data from Appendices 2 and 3 were simplified and recalculated as follows (Table 6.1):

- Data for each series of test cultures were combined (i.e., fresh sediment and enrichment cultures were considered to be equivalent inocula, giving replication of 6 flasks rather than two sets of 3 flasks). This is reasonable because the data for the enrichment culture and fresh sediment inocula were similar for all analyte classes (Tables 5.2 and 5.4). Combining the test culture data focuses attention on the overall biodegradation potential.

- Biodegradation of Bunker C was calculated as the change in analyte mass (mg/g sample) due only to biological processes, and also expressed as a percentage of the original oil added. This differs from calculations in earlier tables, where the percentage of analyte lost was calculated relative to the sterile control analyte. Instead, this calculation shows what percentage of the original Bunker C oil was lost due to biodegradation.

For the 22°C cultures, the TPH loss was only 117 mg/g sample, or only ~12% by weight of the Bunker C added to the flasks (comprising 5.3% as TSH plus 6.4% as TAH). For the 4°C cultures, this was 67.5 mg/g sample, or 6.8% (1.9% as TSH and 4.9% as TAH). That is, an estimated 88 % of the Bunker C oil was not degraded under either incubation condition, given the assumption of no biodegradation of the resins or asphaltenes, which are not detected by GC. This assumption seems valid because no significant

biodegradation of biomarkers was noted (Appendices 2 and 3).

Table 6. 1 Biodegradation losses from Bunker C oil calculated by combining data from test cultures regardless of inoculum source (n=6). Biodegradation is expressed both as mass loss (mean ± 1 standard deviation) due to biological processes and as a percentage of the original Bunker C oil added to the cultures.

Analyte Class	Biodegradation, mg/g sample [a] (mean ± 1 stdev)		% initial of oil biodegraded (mean ± 1 stdev)	
	22°C, 4 wks	4°C, 8 weeks	22°C, 4 wks	4°C, 8 weeks
TPH	**117** ± 19.9	**67.5** ± 13.3	**12**	**6.8**
TSH	**53.1** ± 11.0	**19.2** ± 9.6	**5.3**	**1.9**
TAH	**63.6** ± 13.2	**49.0** ± 5.7	**6.4**	**4.9**
Total *n*-alkanes	**7.8** ± 0.1	**7.2** ± 1.2	**0.8**	**0.7**
Total PAHs	**28.4** ± 2.5	**19.6** ± 3.1	**2.8**	**2.0**

a, Data were compiled and recalculated from Appendices 2 and 3

Expressing the biological losses (biodegradation) as a percentage of the added oil rather than of the amount remaining in the sterile control is a more rigorous way of examining biodegradation potential. In the case of Bunker C oil, it emphasizes how little of the spilled oil is actually being degraded (as detected by GC). It is possible that additional biological losses are occurring in the non-GC-detectable components of the oil, but such studies would require gravimetric analysis of oil losses which are error-prone and beyond the scope of this project.

6.3 Comparison with previous results

Previous biodegradation experiments were conducted with Bunker C oil provided by EC and weathered artificially in the laboratory, resulting in a mass loss of 0.1% (Wang et al. 1998). The inoculum was a defined consortium of oil-degrading microbes derived from fresh water or terrestrial sources (Foght et al., 1998) in the same medium used here but incubated at 10°C for 4 weeks (Wang et al. 1998). Analysis of residual oil was conducted in the same manner except that parallel data for the Total PAH analytes could not be found for the EC dataset, so a sub-set of the Total PAHs, the 5-PAH comprising five homologous series of PAHs, was used for comparison in this table only.

The Lake Wabamun cultures achieved better biodegradation of TPH and signficantly better biodegradation of TAH and 5-PAH in the Wabamun Bunker C oil than the EC standard inoculum did with the EC-Bunker C oil (Table 6.2). Notably, the TAH mass loss from Wabamun oil was 64 mg/g whereas only 8 mg/g was degraded in the EC oil, corresponding to 60% versus 9% biodegradation, relative to the parallel sterile controls. Comparable percentages of the TSH and *n*-alkanes were degraded in both oils by both

inocula (97% and 96% biodegradation), although the mass losses of *n*-alkanes were quite different with only 8 mg/g lost from the Wabamun oil versus 33 mg/g lost from the EC oil. This reflects the initial compositional differences between the two oils, apparent in the Sterile Control flask data, particularly for the *n*-alkanes (Table 6.2). At least two explanations for these differences in biodegradation are possible: the complex microbial population in the lake sediment may be better at degrading aromatics than the three aromatic-degrading strains in the EC standard inoculum, and/or the composition of the Wabamun Bunker C includes a higher proportion of aromatics that are intrinsically more biodegradable than the EC Bunker C oil.

Table 6. 2. Biodegradation of Lake Wabamun Bunker C oil by Lake Wabamun sediment cultures incubated at 22°C for 4 weeks versus biodegradation of EC Bunker C oil by the standard EC freshwater inoculum (Foght et al., 1998) at 10°C for 4 weeks (Wang et al., 1998). Mass loss is expressed as an absolute mass and as a percent of the sterile control for each oil.

	Lake Wabamun cultures Residual Mass, mg/g sample			**EC freshwater inoculum Residual Mass, mg/g sample**		
Analyte Class	Sterile Controls [a]	Test Cultures [b]	Mass loss (%)	Sterile Control [c]	Test Culture [c]	Mass loss (%)
TPH	**239** ± 10	**122** ± 20	**117** (49%)	**303**	**234**	**69** (23%)
TSH	**133** ± 6	**80** ± 11	**53** (40%)	**213**	**155**	**58** (27%)
TAH	**106** ± 4	**42.4** ± 13	**64** (60%)	**91**	**83**	**8** (9%)
Total *n*-alkanes	**8.2** ± 0.5	**0.23** ± 0.13	**8.0** (97%)	**33.8**	**1.3**	**33** (96%)
5-PAHs [d]	**32.3** ± 2.0	**4.2** ± 2.5	**28** (87%)	**18.3**	**10.0**	**8.3** (45%)

a, n=2
b, n=6 (combined data for single Fresh Sediment and Enrichment Culture)
c, n=1
d, Parallel data for Total PAHs could not be found for the Standard EC inoculum so the subset of 5-PAHs (a homologous series of alkylated PAHs and parent compounds) was substituted for both datasets (this table only)

The Bunker C oil recovered from Lake Wabamun apparently is atypical (Wang, pers. com.). Whereas Bunker C oils usually comprise a large proportion of residual fuels with high carbon number constituents (C_{12} to C_{34} alkanes; Lee et al. 2003) and high molecular weight PAHs, such as the 4-ring alkylated chrysenes and higher that represent 5% or more of the total oil weight (Lee et al. 2003), the Bunker C recovered from Lake Wabamun does not. The Carbon number of the Wabamun Bunker C oil ranges primarily from C_{12}-C_{28} with almost no hydrocarbons greater than C_{30}, and low concentrations of the alkylated chrysene series with concomitantly more of the lower weight aromatics, particularly the biodegradable naphthalene and phenanthrene series (see chromatograms and bar graphs in Appendices 2 and 3). The compositional difference between the oils may account for much of the difference in degree of biodegradation observed, and suggests that the Wabamun oil may have a greater proportion of TPH that is

biodegradable than most Bunker C oils. Nonetheless, the TPH is still a small fraction of the total mass of the oil.

7. Recommendations for bioremediation

We designed the experiment to add nutrients (ammonium, nitrate and phosphate) to the incubation medium to ensure that natural concentrations of these nutrients in the sediment did not limit biodegradation. The actual concentration of N and P in Lake Wabamun sediment could be measured and trials conducted to see whether natural levels are limiting. If they are, tests could be performed to determine how much is required and in what chemical formulation to permit maximum biodegradation.

Similarly, we chose to aerate the cultures by agitation. Oxygen is likely limiting beneath the first few mm of undisturbed sediment at Lake Wabamun (note the faint H_2S smell of freshly collected sediment, section 4.1). Mixing of contaminated sediment and water to suspend particulates and oxygenate the water column may accelerate oil biodegradation. Tests could be conducted with static vs. agitated cultures of lake sediment to determine how much effect aeration has on biodegradation. Anaerobic biodegradation of low molecular weight hydrocarbons does occur (e.g., Elshahed et al. 2001) but is much slower than aerobic degradation, appears to be more limited in the range of hydrocarbons that are susceptible to biological attack, and produces organic metabolites that may persist (versus aerobic attack that ideally produces CO_2 and H_2O). Therefore, if at all possible, aerobic conditions should be established to enhance biodegradation of the Bunker C oil.

The observation that the natural Lake Wabamun sediment already contains a high proportion of hydrocarbon-degrading microbes raises the question of whether their abundance is the result of natural chronic low-level contamination or a rapid response to the acute contamination with Bunker C oil. Tests could be conducted using sediment from an uncontaminated area of the lake to determine whether similarly enriched microbial communities also exist outside the area of acute contamination. This might give an indication of the response of clean areas to incursion of Bunker C, should it migrate to previously uncontaminated areas, or whether sediment from a contaminated site at the lake could be used as a natural "inoculum" to accelerate remediation of the newly impacted site.

However, given the chemical nature of Bunker C oil, and the small proportion of compounds that appear to be "readily biodegradable", intervention such as nutrient amendment, aeration and inoculation will only accelerate removal of the minor biodegradable portion of the oil (possibly less than 12% by mass), with the residual oil being more recalcitrant and more likely to persist.

Another consideration is microbial access to the spilled oil. The Bunker C used in the laboratory tests was recovered from the lake as discrete floating and sinking "tar balls". The occurrence of these "tar balls" (i.e., discrete globs of oil with a weathered "rind" of

material that has been oxidized by exposure to sun and volatilization) will increase the recalcitrance of the oil inside the balls. Microbial access to the biodegradable components remaining inside the balls will decrease because of the low surface area:volume ratio (versus small droplets with high surface area) and because the surface skin presents a poorly degradable substratum for microbes to attach to. Therefore even the labile components inside the balls may remain undegraded. It is unlikely that the use of surfactants would overcome this problem.

It is also noted that the Bunker C oil balls used in testing appeared to be less weathered (i.e., retains more of the lower molecular weight, biodegradable constituents) than many other forms of submerged oil in the lake. It may be inferred that degradation of the other forms of remaining oil would be even slower than those tested.

Overall, the prognosis for extensive natural bioremediation over the short term, say 5 – 10 years, is poor, and these laboratory tests indicate that a large proportion of the spilled oil will persist even under optimum conditions for biodegradation.

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9. Appendices

Appendix 1: Composition of Media and Solutions

Bushnell-Haas Broth (Atlas 1995), per Litre of doubly distilled water:
1g K_2HPO_4; 1 g KH_2PO_4; 0.2 g $MgSO_4.7H_2O$; 0.02 g $CaCl_2.2H_2O$; 0.05 g $FeCl_3$; 1 g NH_4NO_3, pH 7

Freshwater medium (Foght et al. 1998), per Litre of doubly distilled water: 0.5 g K_2HPO_4; 2 g Na2SO4; 0.2 g $MgSO_4.7H_2O$; 0.75 g KCl; trace $CaCl_2.2H_2O$; trace $FeSO_4$; 1 mL 1000X trace metals solution; pH 7. Add XX mL Standard N,P Nutrient solution to sterile, cooled medium.

Standard (Westlake) N,P Nutrient Solution (Foght et al. 1998) (50X stock) per Litre of doubly distilled water: 50 g NH_4Cl; 100 g KNO_3; 25 g K_2HPO_4, Add 4 ml/200 mL

1000X Trace Metals solution (Fedorak and Grbic -Galic 1991), per Litre of doubly distilled water: 3.7 g $CaCl_2.2H_2O$; 2.5 g H_3BO_3; 0.87 g $MnCl_2$; 0.65 g $FeCl_3$; 0.44 g $ZnCl_2$; 0.29 g $Na_2MoO_4.2H_2O$; 0.01 g $CoCl_2$; 0.0001 g $CuCl_2$.

Appendix 2: ESTD-ETC report 2006-04

(Attached electronically)

Appendix 3: ESTD-ETC report 2006-05

(Attached electronically)

Memorandum

Report: 2006-04

Date: May 25, 2006

To: Gary Sergy
Head, Prarie and Northern Section

Julia Foght, Ph. D
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Merv Fingas
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Subject: **Characterization of the Biodegraded Wabamun Spill Oil Samples (1st Batch) Incubated with Lake Wabamun Sediment or Wabamun Enrichment Culture at 22 ^{0}C for 28 Days**

On August 3, 2006, a CN (Canadian National Railway) train derailment occurred within Whitewood Sands community of the Wabamun Lake area, in the province of Alberta. The derailment resulted in the release of 712,500 liters of Bunker C oil and 88,000 liters of Imperial Pole Treating oil (PTO) from the train cars onto the ground in the vicinity of the derailment and then flowed into the water of the adjacent Wabamun Lake. Remediation activities were initiated at the derailment site on the same day that the derailment occurred. Remediation activities were initiated at the derailment site on the same day that the derailment occurred.

After the accident, Environment Alberta and Environment Canada (EC) conducted a number of sampling programs at the spill site for research on the fate, behavior and impact of the spilled oil. At the selected site, observations and measurements were made, and a significant number of samples (including relatively "fresh" oils from the pools near the overturned rail tanks, oil contaminated water and sediment samples) was collected for petroleum hydrocarbon analyses in order to quantitatively determine the chemical composition of the spilled oil in contaminated

samples.

Biodegradation of oil hydrocarbons by natural population of microbial represents one of the primary mechanisms by which oil and oil-related hydrocarbons are eliminated from the contaminated environment. In order to determine the biodegradation potential of the Wabamun spilled Bunker C oil, a biodegradation study was conducted by University Alberta and Environment Canada. The biodegradation data obtained from this study will be used to make judicious decisions regarding clean-up effort and the potential use of bioremediation strategies in the contaminated sites.

1. Oils

There are two oils used for the biodegradation study and for oil biodegradation potential assessment: the Wabamun spilled Bunker C oil (recovered from the spill site) and the Alberta Sweet Mixed Blend (ASMB). The ASMB oil, which has been well demonstrated to be biodegradable, was included in the study as a positive control to prove that hydrocarbon-degrading bacteria were present in the sediment and enrichment culture.

2. Biodegradation Method

Eleven weathered Wabamun oil and ASMB oil flask were incubated with Lake Wabamun Fresh Sediment or Lake Wabamun Enrichment culture (Table 1) for biodegradation potential assessment. The "Enrichment" (which was produced from the oil-contaminated sediment from Lake Wabamun) comprises a mixed population of microbes, presumably with a higher proportion of hydrocarbon-degrading microbes that are adapted to growth on the Wabamun oil.

The Wabamun oil recovered from the spill site was dissolved in about 10X volume of spectrophotometric-grade dichloromethane (DCM) and passed through a 0.22 µm Whatman filter to remove sand grass and water, and then filter sterilized through a 0.22 µm Millex-FG filter. The DCM solution (5mL = 0.392 g) was distributed into each flask containing sterilized medium. The flasks were shaken at room temperature for 48 hours to remove any DCM before inoculation with lake mud slurry or enrichment culture. The ASMB oil was directly added gravimetrically to flasks, using a 5 mL ground glass syringe without any prior dilution, weighed before and after each oil addition to nearest 0.1 mg. Duplicate, uninoculated flasks served as sterile "weathering" controls to account for abiotic oil losses through volatility during the incubation period. The ASMB oil (our standard reference oil), as indicated above, was incubated with each batch of 3 Wabamun oils tested, for quality control. All flasks of Fresh Sediment, Enrichment Culture, and sterile controls were incubated at 22 ^{0}C in darkness with shaking for 28 days. After incubation, 1.0 mL of surrogate standards solution (o-terphenyl, d10-phenanthrene, and squalane) was added to the flasks, which were then acidified and extracted exhaustively

with DCM. The extracts were dried by passage through anhydrous sodium sulfate, concentrated to small volumes on a rotovap, and then transferred to tared vials and blown down to dryness using gentle N_2 stream to calculate gravimetric recovery of tested oils.

Table 1 Wabamun oil degradation experiment
(22 ^{0}C, 28 days; set up on January 25, 2006)

Sample type	Sample (ESTD #)	Oil type	Weigh oil added (g)	Wt. Vial (g)	Wt. Vial + oil (g)	Wt. Oil recovered* (g)	% recovered
Wab Fresh Sediments	773	Wab 1	0.392	4.8524	5.2255	0.3731*	95
	774	Wab 1	0.392	4.8387	5.1519	0.3132*	80
	775	Wab 1	0.392	4.8230	5.2134	0.3904*	100
	776	ASMB	0.4084	4.8606	5.0506	0.1900*	47
Wab Enrichment Culture	777	Wab 1	0.392	4.8453	5.2318	0.3865*	99
	778	Wab 1	0.392	4.8323	5.2206	0.3883*	99
	779	Wab 1	0.392	4.8517	5.2562	0.4045*	103
	780	ASMB	0.399	4.8378	5.0334	0.1956*	49
Sterile Control with Sediments	781	Wab 1	0.392	4.8494	5.4195	0.5701*	145
	782	Wab 1	0.392	4.8838	5.3757	0.4919*	125
	783	ASMB	0.4174	4.7408	5.0241	0.2833*	68

** Note: It is found that significant amounts of observable fines were entrained in the oil sample.*

3. Sample Preparation and Fractionation

The dried extracted oils recovered from the biodegradation test were dissolved in hexane (to help dissolution, 0.1 mL of DCM was added) and made up to the final volume of 5.00 mL.

The ESTD reference oil (Prudhoe Bay oil, 13% weathered) was used as the reference oil standard for quality control.

200 µL of the oil solutions was spiked with appropriate surrogates (100 µL 200 ppm of o-terphenyl and 100 µL of mixture of deuterated acenaphthene, phenanthrene, benz[a]anthracene, and perylene, 10 ppm each), and then quantitatively transferred into a 3-g silica gel microcolumns, which was topped with about 1-cm anhydrous granular sodium sulfate and had been pre-conditioned using 20 mL of hexane, for sample cleanup and fractionation.

Hexane (12 mL) and 50% DCM in hexane (v/v, 15 mL) were used to elute the saturated and aromatic hydrocarbons, respectively. For each sample, half of the hexane fraction (labeled F1) was used for analysis of aliphatics, n–alkanes, and sesquiterpanes and biomarker terpane and sterane

compounds; half of the 50% benzene fraction (labeled F2) was used for analysis of alkylated homologous PAHs and other EPA priority unsubstituted PAHs; the remaining halves of the hexane fraction and 50% benzene fraction were combined into a fraction (labeled F3) and used for the determination of the total GC-detectable petroleum hydrocarbons (TPH) and the unresolved complex mixture of hydrocarbons (UCM). These three fractions were concentrated under a gentle stream of nitrogen to appropriate volumes (~0.6 mL), spiked with appropriate internal standards (100 µL of 200 ppm 5-α-androstane, 100 µL of 10 ppm cis-decahydronaphthalene-d_{18} and 100 µL of 10 ppm C_{30}-ββ-hopane; 100 µL of 10 ppm terphenyl-d_{14}; and 100 µL of 200 ppm 5-α-androstane for F1, F2, and F3 respectively), and then adjusted to an accurate pre-injection volume of 1.00 mL for GC/MS and GC/FID analyses.

4. Instruments and Sample Analysis

Analyses for the total petroleum hydrocarbons (TPH), total-saturates, total aromatics, and *n*-alkane distribution (n-C_8 through n-C_{41}, pristane and phytane) were performed on an Agilent 6890 gas chromatograph equipped with a flame-ionization detector (FID) and a 7683 series autosampler. A 30 m x 0.32 mm i.d. (0.10 µm film thickness) DB-5HT (high temperature) fused-silica capillary column was used. The carrier gas was hydrogen (2.5 mL/min). The injector and detector temperature was set at 290 and 325 ^{0}C. The oven temperature program employed was: 2 min hold at 40 ^{0}C, ramp to 340 ^{0}C at 25 ^{0}C /min, and 15 minute hold at 340 ^{0}C. A 1 µL aliquot was injected in the splitless mode with a 1-min purge-off. GC-FID analysis provides a baseline resolution of n-alkanes from n-C_8 to n-C_{41}. Quantitation of the analytes was based on the internal standard compound (5-α-androstane).

Characterization and quantitation of target PAH compounds (including 5 alkylated PAH homologous groups and other EPA priority PAHs) and biomarker compounds were performed on an Agilent 6890 GC equipped with an Agilent 5973 mass selective detector (MSD). System control and data acquisition were achieved with an HP G1701 BA MSD ChemStation. A 30 m x 0.32 mm i.d. (0.25 µm film thickness) HP-5MS fused-silica capillary column is used. The chromatographic conditions are as follows: carrier gas, helium (1.0 mL/min); injection mode, spiltless; solvent delay, 4 minutes; injector temperature, 280 °C; MSD heater and source temperature, 280 and 230 °C, respectively. The temperature program employed for biomarkers and alkylated PAHs is: 50 °C hold for 2 min, then ramp at 6 °C/min to 300 °C and hold for 17 minutes. Prior to sample analysis, the GC-MS was tuned with perfluorotributylamine (PFTBA). The concentrations of the individual PAH and biomarker compounds were determined based on the internal standards d_{14}-terphenyl and C_{30}-ββ-hopane, respectively. The bicyclic sesquiterpanes were determined based

on the IS cis-decahydronaphthalene-d_{18}. For more details on analysis quality control and quantification methodology, refer to references by Wang et al. (Wang et al., 1994a, 1994b, and 2003).

The RRFs for individual PAH compound quantitation were determined during instrument calibration. The average RRF for the biomaker compound C_{30} 17b(H) 21a(H)-hopane was determined relative to the internal standard C_{30} 17b(H) 21b(H)-hopane. The average RRF for C_{30} 17b(H) 21a(H)-hopane (m/z 191) was used for quantitation of C_{30} 17b(H) 21b(H)-hopane and other triterpanes in the oil sample. For quantitation of steranes, the RRF of C_{29} 20R aaa-ethylcholestane monitored at m/z 217 was determined relative to C_{30} 17b(H) 21b(H)-hopane monitored at m/z 191, and then the average RRF of C_{29} 20R aaa-ethylcholestane was used for determination of sterane compounds in the oil.

5. Characterization Results and Discussion

(1) Determination of Hydrocarbon Groups and n-Alkane Distribution

Figures 1, 2 and 3 show the GC/FID chromatograms of F1, F2 and F3 for total saturates, total aromatics, and total petroleum hydrocarbon (TPH) analyses, respectively. Comparison of the GC chromatograms immediately reveals that biodegradation had been occurred during 28 days inoculation.

Table 2 summarizes the hydrocarbon group quantitation results of the samples. Table 3 summarizes the n-alkane values in biodegraded samples. Figure 4 depicts graphically the n-alkane distribution in biodegraded samples. *Note that all calculations were based on oil weight added, not on oil weight recovered.*

(1) Significantly degradation was demonstrated for the ASMB oil. The TPH values were determined to be 415, 152, and 196 mg/g sample for the ASMB sterile control, ASMB Fresh sediment, and ASMB Enrichment culture samples, respectively. The concentrations of the total saturates and total aromatics in biodegraded ASMB oil samples were considerably reduced too. n-Alkanes showed even greater degradation: n-alkanes including isoprenoids pristine and phytane were nearly completely gone in comparison to 69 mg n-alkanes per gram of sample for the ASMB oil sterile control. These facts clearly proved that hydrogen-degrading bacteria were present in the sediment and enrichment culture and were effective for oil hydrocarbon degradation.

It has demonstrated that GC-resolved peaks degraded faster than the unresolved complex mixture of hydrocarbons. The ratios of total resolved peaks/TPH were determined to be 41, 11.8, and 9.9 for the ASMB sterile

control, ASMB Fresh sediment, and ASMB Enrichment culture samples, respectively.

(2) For all biodegraded Wabamun oil samples, TPH, total-saturates and total-aromatics showed degradation to various degrees. The TPH values were determined to be in the range of 88-140 mg/g of sample (n=3, mean=114 and 131 mg/g for the Fresh sediment, and Enrichment culture, resepectively) for the Wabamun biodegraded oil samples. For the Wabamun sterile controls, the average TPH value was determined to be 239 mg/g of sample (n=2).

(3) n-Alkanes and isoprenoids were nearly completely degraded in both Wabamun fresh sediment and enrichment culture samples. No n-alkanes with the carbon number smaller than C_{23} were detected.

The averages of the total n-alkanes were determined to be 8.2 mg/g sample for the Wabamun sterile control (n=2), 0.33 (0.24, 0.43, and 0.31 for three replicate fresh sediment samples) and 0.13 (0.15, 0.18, and 0.07 for three replicate enrichment culture samples) mg/g for the Fresh sediment and Enrichment culture samples (n=3), respectively.

(2) Determination of Target Alkylated PAH Homologues and Their Diagnostic Ratios

Figure 5 shows the total ion GC/MS chromatograms (in SIM mode) of the samples for analyses of target alkylated PAH homologues. Table 4 summarizes quantitation results of 5 petroleum-characteristic alkylated PAH homologous series (alkylated naphthalene, phenanthrene, dibenzothiophene, fluorene, and chrysene series) and other EPA priority PAHs. For comparison, diagnostic ratios of some target PAH groups and paired isomers are also presented in Table 4. Figure 6 graphically depicts the distribution of these target PAH compounds.

(1) Aromatic compounds including 5-target alkylated PAH homologous series were significantly degraded.

The total alkylated PAHs were determined to be 10671, 309 and 421 μg/g of sample for ASMB sterile control, ASMB-fresh sediment, and ASMB-enrichment culture, respectively. It is apparent that the concentrations of target PAHs in degraded oil samples were dramatically reduced due to biodegradation.

The total alkylated PAHs were determined to be 32570 (mean of 33967 and 31172) μg/g of oil sample for the Wabamun oil sterile controls. In contrast, the total alkylated PAHs were determined to be only 4754 and 3661 μg/g of sample (in the range of 2000 to 8600 μg/g of sample, n=3) for

the Wabamun oil fresh sediment and enrichment culture biodegradation samples. Degradation of Wabamun oil PAHs is clearly demonstrated.

(2) Among the five target alkylated PAH homologues, the alkyl homologues of naphthalene (the most abundant PAHs. 2-ring) were the most degraded series (80-95% of alkylated naphthalenes have been degraded), followed by alkyl homologous series of dibenzothiophene, fluorine, and then phenanthrene. In comparison with the sterile controls, however, the concentrations of the alkylated chrysenes (4-rings) in most Wabamun oil samples were more or less increased due to their higher biodegradation-resistant property.

Among other EPA priority PAHs, low molecular weight PAHs (including biphenyl, acenaphthalene, acenaphthene, and anthracene) were nearly completely lost, while 5-6 ring PAHs were shown to be much less degraded or even enriched due to their high degradation-resistant properties and due to reduction of the total oil volumes by biodegradation).

(3) An increase in alkylation level clearly decreases susceptibility to microbial attack, resulting in the distribution pattern of C0- < C1- < C2- < C3- in each alkylated PAH homologous series.

(4) GC-resolved aromatics degraded, in general, faster than unresolved aromatic hydrocarbons, indicated by much higher percentages of the 5 target alkylated PAH homologues lost than the total aromatics.

(5) PAH isomers in the same alkylation levels demonstrated to be biodegradation-specific. For example, the diagnostic ratios of 3 methyl-dibenzothiophenes, 2 methyl-naphthalenes, and 4 methyl-phenanthrenes in the Wabamun oil samples were determined to be significantly different from the corresponding values for the sterile controls (Table 4).

(3) Characterization of Terpane and Sterane Biomarkers

The biomarker characterization results clearly demonstrated there was no observable sigh of alteration of biomarker terpane and steranes (detailed results are not shown here). For example, the total of the target biomarker terpanes and steranes were determined to be 890 μg/g sample for the ASMB oil sterile control, 898 and 916 μg/g sample for the fresh sediment and enrichment culture of ASMB oil samples respectively. Concentrations of individual biomarkers were almost not altered.

(4) Percentage Loss of Hydrocarbon Groups

Table 5 summarizes the quantitative composition changes and percentage loss of hydrocarbon groups in 11 tested biodegradation oil samples.

(1) Biodegradation of the Wabamun spill oil by microbial is clearly demonstrated. Compared with the Wabamun sterile control, 52.3% and 45.2% of the total GC-detectable hydrocarbons were determined to be lost by biodegradation for the fresh sediment and enrichment culture oil samples, respectively.

(2) It is noted that the Wabamun fresh sediment samples showed significantly greater biodegradation of aromatics than the Wabamun enrichment culture samples (35 mg/g over 50 mg/g of sample) under the current biodegradation conditions (22 ^{0}C, 4 weeks), indicating that "enrichment" may not contain a higher proportion of aromatic hydrocarbon-degrading microbes and it did not enhance biodegradation of Wabamun spill oil samples in whole.

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Table 2. GC-TPH analysis results

Sample	Wabamun fresh sediment, 22C, 4 weeks					Wabamun Enrichment culture, 22C, 4 weeks					Wabamun Sterile Control with Sediment, 22C, 4 weeks				ASMB, 22C, 4 weeks	ASMB Enrichment culture, 22C, 4 weeks	ASMB Sterile Control, 22C, 4 weeks
ESTD Log No.	2006/03/02-0773	2006/03/02-0774	2006/03/02-0775	Mean	RSD	2006/03/02-0777	2006/03/02-0778	2006/03/02-0779	Mean	RSD	2006/03/02-0781	2006/03/02-0782	Mean	RSD	2006/03/02-0776	2006/03/02-0780	2006/03/02-0783
Client Sample No.	#1	#2	#3		(%)	#9	#10	#11		(%)	#17	#18		(%)	#4	#12	#19
TPH (mg/g sample)	115	88.5	137	114	21.4	131	118	144	131	9.8	246	232	239	4.0	152	196	415
TSH (mg/g sample)	89.5	60.5	87.2	79.0	20.4	76.7	77.4	88.1	80.7	8.0	137	129	133	4.2	109	114	274
TAH (mg/g sample)	25.7	28.1	49.9	34.5	38.5	54.7	40.6	55.6	50.3	16.7	109	103	106	3.8	43.3	81.6	141
TSH/TPH (%)	77.7	68.3	63.6	69.9	10.3	58.4	65.6	61.3	61.7	5.9	55.6	55.5	55.6	0.2	71.6	58.3	66.1
TAH/TPH (%)	22.3	31.7	36.4	30.1	23.8	41.6	34.4	38.7	38.3	9.5	44.4	44.5	44.4	0.2	28.4	41.7	33.9
Resolved peaks/TPH (%)	14.5	14.1	18.8	15.8	16.5	17.7	16.1	18.8	17.5	7.7	40.6	41.3	41.0	1.2	11.8	9.90	41.2

* TSH: total saturated hydrocarbons; TAH: total aromatic hydrocarbons.

Table 3 *n*-Alkanes anaylsis results

Sample	Wabamun fresh sediment, 22C, 4 weeks			Wabamun Enrichment culture, 22C, 4 weeks			Wabamun Sterile Control with Sediment, 22C, 4 weeks		ASMB, 22C, 4 weeks	ASMB Enrichment culture, 22C, 4 weeks	ASMB Sterile Control, 22C, 4 weeks
ESTD Log No.	2006/03/02-0773	2006/03/02-0774	2006/03/02-0775	2006/03/02-0777	2006/03/02-0778	2006/03/02-0779	2006/03/02-0781	2006/03/02-0782	2006/03/02-0776	2006/03/02-0780	2006/03/02-0783
Client Sample No.	#1	#2	#3	#9	#10	#11	#17	#18	#4	#12	#19
n-Alkanes (mg/g sample)											
C_8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C_9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C_{10}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09
C_{11}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72
C_{12}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.65
C_{13}	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.04	0.00	0.00	2.60
C_{14}	0.00	0.00	0.00	0.00	0.00	0.00	0.36	0.27	0.00	0.00	3.37
C_{15}	0.00	0.00	0.00	0.00	0.00	0.00	0.76	0.64	0.00	0.00	3.69
C_{16}	0.00	0.00	0.00	0.00	0.00	0.00	0.86	0.76	0.00	0.00	3.35
C_{17}	0.00	0.00	0.00	0.00	0.00	0.00	0.96	0.88	0.00	0.00	3.49
Pristane	0.00	0.00	0.00	0.00	0.00	0.00	0.36	0.36	0.00	0.00	2.03
C_{18}	0.00	0.00	0.00	0.00	0.00	0.00	0.87	0.80	0.00	0.00	2.80
Phytane	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.31	0.00	0.00	1.81
C_{19}	0.00	0.00	0.00	0.00	0.00	0.00	0.87	0.82	0.00	0.00	2.47
C_{20}	0.00	0.00	0.00	0.00	0.00	0.00	1.01	0.92	0.00	0.00	2.42
C_{21}	0.00	0.00	0.00	0.00	0.00	0.00	0.75	0.69	0.00	0.00	2.08
C_{22}	0.00	0.00	0.00	0.00	0.00	0.00	0.49	0.44	0.00	0.00	1.94
C_{23}	0.00	0.03	0.03	0.00	0.00	0.00	0.29	0.26	0.00	0.00	1.67
C_{24}	0.03	0.03	0.01	0.01	0.01	0.00	0.14	0.12	0.00	0.00	1.40
C_{25}	0.06	0.07	0.05	0.04	0.03	0.00	0.10	0.13	0.00	0.00	1.66
C_{26}	0.02	0.04	0.02	0.02	0.01	0.01	0.04	0.04	0.00	0.00	1.37
C_{27}	0.05	0.06	0.03	0.02	0.01	0.01	0.10	0.10	0.00	0.00	1.22
C_{28}	0.01	0.04	0.02	0.02	0.02	0.01	0.03	0.03	0.00	0.00	1.00
C_{29}	0.02	0.04	0.03	0.01	0.02	0.01	0.04	0.05	0.00	0.00	1.04
C_{30}	0.01	0.03	0.02	0.01	0.02	0.01	0.02	0.02	0.00	0.00	0.68
C_{31}	0.01	0.02	0.02	0.01	0.02	0.01	0.03	0.04	0.00	0.00	0.63
C_{32}	0.01	0.02	0.02	0.01	0.02	0.01	0.02	0.02	0.00	0.00	0.57
C_{33}	0.01	0.01	0.01	0.00	0.01	0.01	0.02	0.02	0.00	0.00	0.33
C_{34}	0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.31
C_{35}	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.17
C_{36}	0.00	0.01	0.01	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.14
C_{37}	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.11
C_{38}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.05
C_{39}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
C_{40}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
C_{41}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C_{42}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C_{43}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C_{44}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	**0.24**	**0.43**	**0.31**	**0.15**	**0.18**	**0.07**	**8.57**	**7.80**	**0.00**	**0.00**	**48.8**
Mean		**0.33**			**0.13**			**8.18**			
Diagnostic Indexes											
C_{17}/Pristane	NA	NA	NA	NA	NA	NA	2.67	2.48	NA	NA	1.72
C_{18}/Phytane	NA	NA	NA	NA	NA	NA	2.72	2.61	NA	NA	1.54
Pr/Ph	NA	NA	NA	NA	NA	NA	1.12	1.17	NA	NA	1.12
Odd alkanes	0.14	0.25	0.19	0.09	0.09	0.03	4.02	3.68	0.00	0.00	21.8
Even alkenes	0.09	0.18	0.12	0.07	0.09	0.03	3.86	3.45	0.00	0.00	21.2
CPI	1.59	1.40	1.52	1.28	0.98	0.88	1.04	1.07	NA	NA	1.03

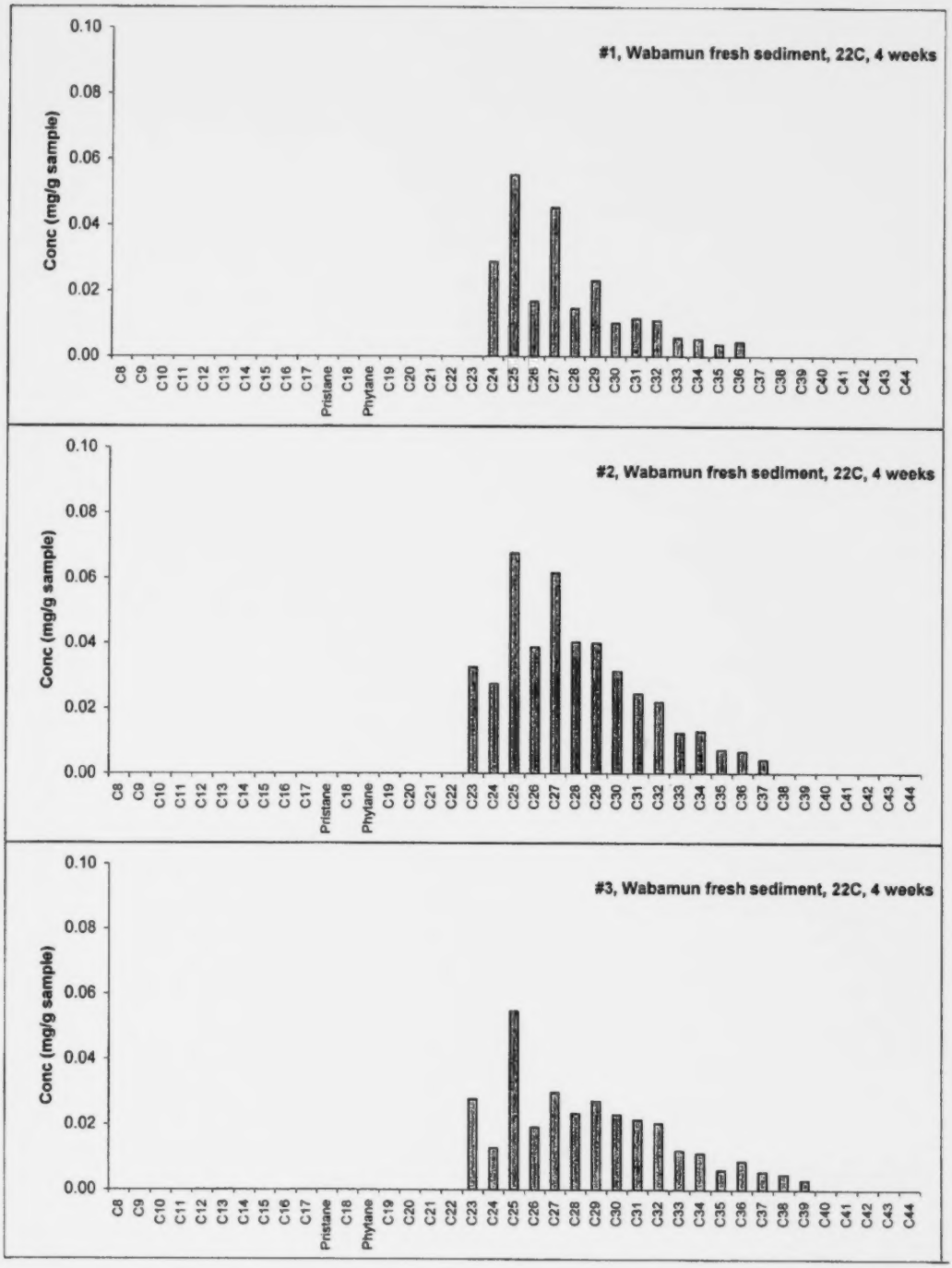
#1, Wabamun fresh sediment, 22C, 4 weeks
#2, Wabamun fresh sediment, 22C, 4 weeks
#3, Wabamun fresh sediment, 22C, 4 weeks
Conc (mg/g sample)
0.10
0.08
0.06
0.04
0.02
0.00
C8 C9 C10 C11 C12 C13 C14 C15 C16 C17 Pristane C18 Phytane C19 C20 C21 C22 C23 C24 C25 C26 C27 C28 C29 C30 C31 C32 C33 C34 C35 C36 C37 C38 C39 C40 C41 C42 C43 C44

Figure 4 n-Alkane distribution

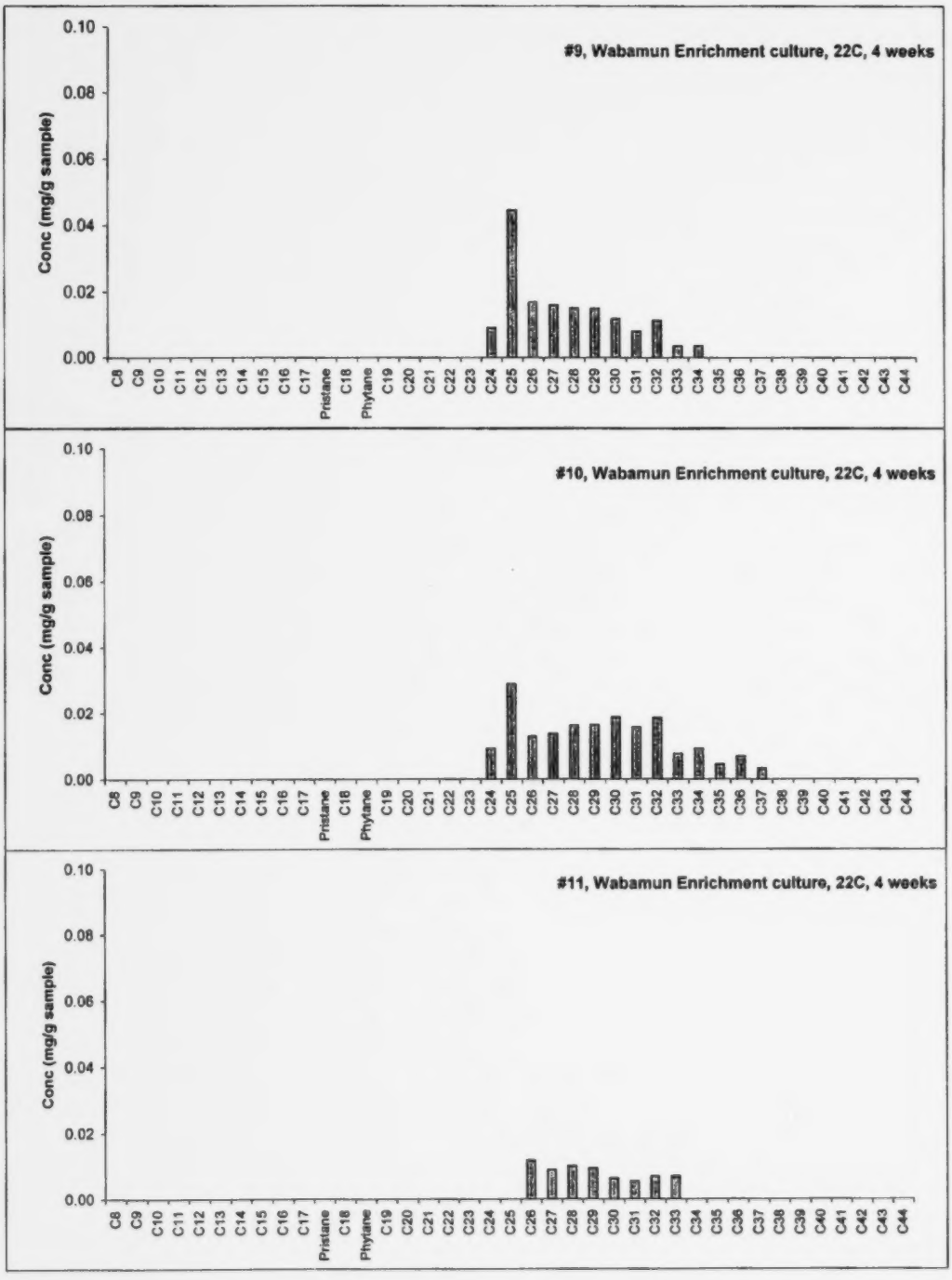
#9, Wabamun Enrichment culture, 22C, 4 weeks
#10, Wabamun Enrichment culture, 22C, 4 weeks
#11, Wabamun Enrichment culture, 22C, 4 weeks
Conc (mg/g sample)
0.10
0.08
0.06
0.04
0.02
0.00
C8 C9 C10 C11 C12 C13 C14 C15 C16 C17 Pristane C18 Phytane C19 C20 C21 C22 C23 C24 C25 C26 C27 C28 C29 C30 C31 C32 C33 C34 C35 C36 C37 C38 C39 C40 C41 C42 C43 C44

Figure 4 n-Alkane distribution

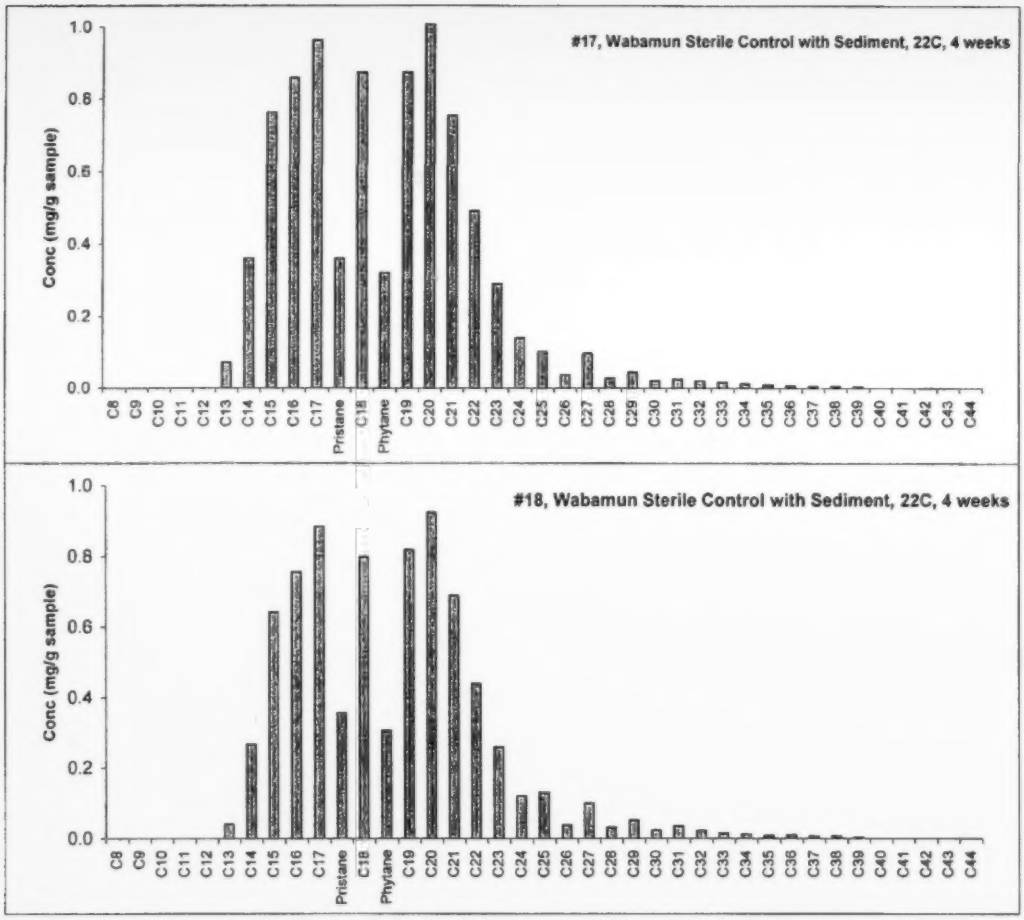
#17, Wabamun Sterile Control with Sediment, 22C, 4 weeks
Conc (mg/g sample)
1.0
0.8
0.6
0.4
0.2
0.0
C8 C9 C10 C11 C12 C13 C14 C15 C16 C17 Pristane C18 Phytane C19 C20 C21 C22 C23 C24 C25 C26 C27 C28 C29 C30 C31 C32 C33 C34 C35 C36 C37 C38 C39 C40 C41 C42 C43 C44
#18, Wabamun Sterile Control with Sediment, 22C, 4 weeks
Conc (mg/g sample)
1.0
0.8
0.6
0.4
0.2
0.0
C8 C9 C10 C11 C12 C13 C14 C15 C16 C17 Pristane C18 Phytane C19 C20 C21 C22 C23 C24 C25 C26 C27 C28 C29 C30 C31 C32 C33 C34 C35 C36 C37 C38 C39 C40 C41 C42 C43 C44

Figure 4 n-Alkane distribution

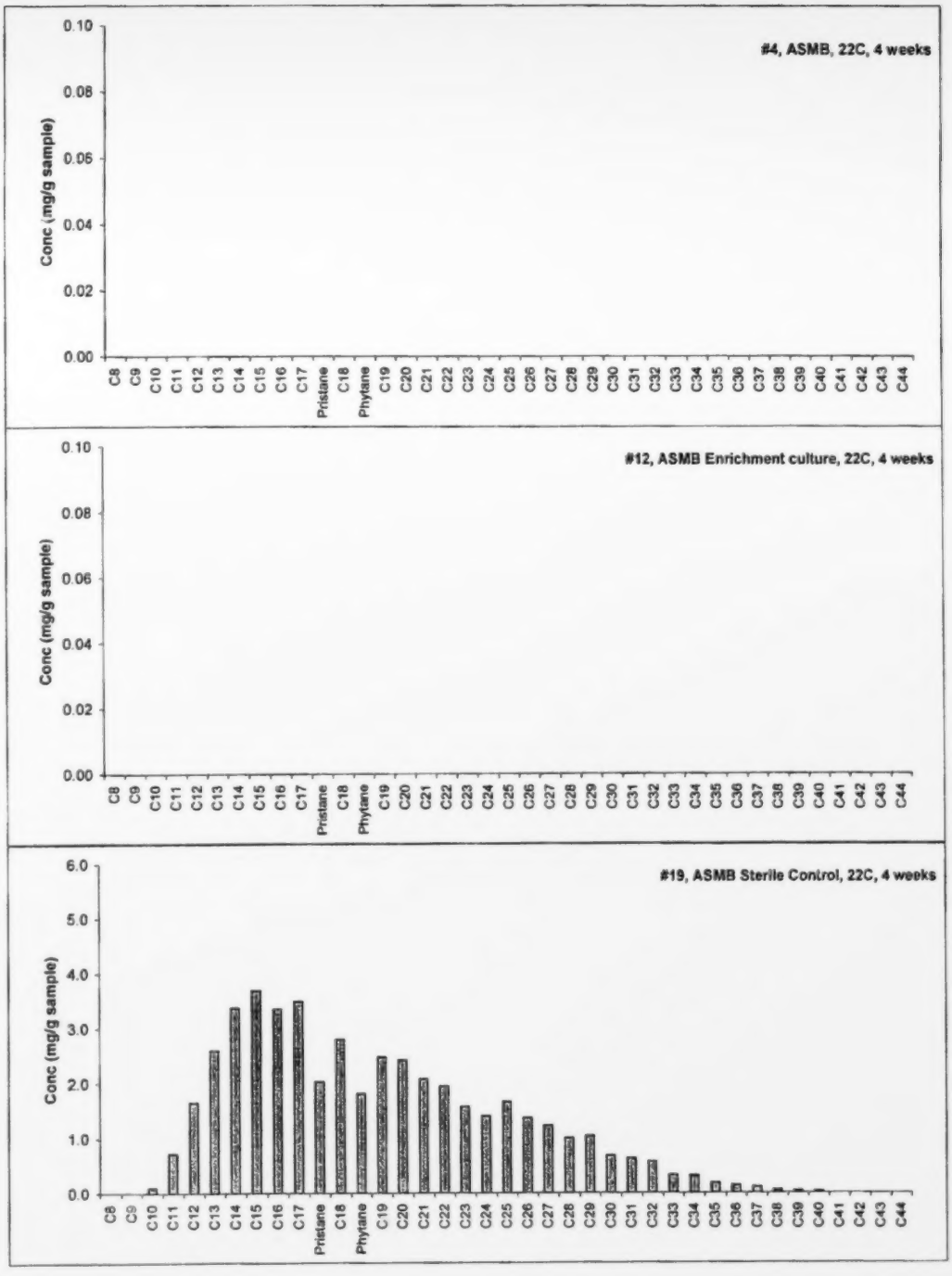
#4, ASMB, 22C, 4 weeks
#12, ASMB Enrichment culture, 22C, 4 weeks
#19, ASMB Sterile Control, 22C, 4 weeks
Conc (mg/g sample)
0.00
0.02
0.04
0.06
0.08
0.10
1.0
2.0
3.0
4.0
5.0
6.0
C8 C9 C10 C11 C12 C13 C14 C15 C16 C17 Pristane C18 Phytane C19 C20 C21 C22 C23 C24 C25 C26 C27 C28 C29 C30 C31 C32 C33 C34 C35 C36 C37 C38 C39 C40 C41 C42 C43 C44

Figure 4 n-Alkane distribution

Table 4 PAHs analysis results

Sample		Wabamun fresh sediment, 22C, 4 weeks			Wabamun Enrichment culture, 22C, 4 weeks			Wabamun Sterile Control with Sediment, 22C, 4 weeks		ASMB, 22C, 4 weeks	ASMB Enrichment culture, 22C, 4 weeks	Control, 22C, 4 weeks
ESTD Log No.		2006/03/02-0773	2006/03/02-0774	2006/03/02-0775	2006/03/02-0777	2006/03/02-0778	2006/03/02-0779	2006/03/02-0781	2006/03/02-0782	2006/03/02-0776	2006/03/02-0780	2006/03/02-0783
Client Sample No.		#1	#2	#3	#9	#10	#11	#17	#18	#4	#12	#19
Alkylated PAHs (µg/g sample)												
Naphthalene	C0-N	0.96	0.96	1.12	0.95	0.88	0.99	1.25	1.07	1.20	1.34	54.8
	C1-N	16.7	6.04	3.35	0.70	0.46	2.99	346	283	7.44	2.54	703
	C2-N	86.7	77.7	108	2.38	1.79	88.1	3851	3396	14.7	4.94	1760
	C3-N	240	232	867	15.6	7.49	477	7345	6716	14.9	5.03	2041
	C4-N	657	478	1670	1186	562	1784	5174	4866	40.9	31.4	1235
	Sum	**1001**	**795**	**2650**	**1206**	**572**	**2333**	**16718**	**15263**	**79.2**	**45.3**	**5794**
Phenanthrene	C0-P	8.57	13.3	140	0.97	0.74	31.4	1419	1291	1.69	0.47	167
	C1-P	209	220	955	7.71	21.3	245	3826	3559	7.12	2.25	502
	C2-P	452	364	1197	364	259	620	2520	2328	21.3	4.28	621
	C3-P	202	148	403	436	295	479	672	601	25.2	11.24	478
	C4-P	58.5	42.5	108	142	121	144	157	154	19.3	78.64	294
	Sum	**948**	**788**	**2802**	**950**	**697**	**1520**	**8593**	**7934**	**74.5**	**96.9**	**2062**
Dibenzothiophene	C0-D	3.82	3.97	34.7	5.06	2.84	13.7	331	306	0.64	1.50	145
	C1-D	64.0	63.4	269	18.3	11.2	67.0	1082	984	3.86	2.08	334
	C2-D	248	195	622	144	112	287	1294	1187	12.3	0.67	524
	C3-D	159	122	338	242	189	247	542	481	17.3	4.17	397
	Sum	**475**	**385**	**1264**	**409**	**315**	**615**	**3230**	**2958**	**34.0**	**8.41**	**1400**
Fluorene	C0-F	2.11	2.28	9.43	1.98	0.76	6.95	233	210	0.34	0.14	60.4
	C1-F	31.6	29.1	170	61.0	24.2	158	1081	990	2.30	1.74	201
	C2-F	240	186	757	144	102	427	2105	1934	7.80	6.88	394
	C3-F	372	267	840	307	236	506	1653	1559	24.5	7.90	422
	Sum	**646**	**484**	**1777**	**514**	**362**	**1099**	**5072**	**4693**	**34.9**	**16.7**	**1077**
Chrysene	C0-C	3.91	3.27	6.39	6.74	5.75	6.55	8.51	7.48	8.97	20.2	30.4
	C1-C	13.2	9.5	19.0	25.2	21.7	26.3	26.5	27.2	17.0	36.0	62.4
	C2-C	21.3	17.1	30.4	39.9	35.9	35.5	39.0	35.8	20.3	88.3	93.3
	C3-C	17.4	12.8	20.6	29.2	25.6	25.2	25.5	23.1	26.8	85.7	69.4
	Sum	**55.8**	**42.6**	**76.4**	**101**	**89.0**	**93.5**	**99.5**	**93.6**	**75.1**	**230**	**256**
Total alkylated PAHS		**3117**	**2495**	**8589**	**3180**	**2036**	**5661**	**33712**	**30941**	**298**	**397**	**10588**
Other Priority PAHs (µg/g sample)												
Biphenyl		1.01	0.85	0.35	0.03	0.04	0.17	22.8	19.5	0.38	0.10	38.0
Acenaphthylene		0.27	0.50	0.52	0.49	0.19	0.33	19.2	16.5	0.04	0.00	6.77
Acenaphthene		1.13	0.78	1.50	0.00	0.00	1.66	61.6	57.3	0.10	0.01	5.83
Anthracene		1.95	1.69	13.0	0.00	0.00	0.00	101	94.1	0.18	0.00	4.18
Fluoranthene		1.67	1.77	5.89	0.44	0.00	0.00	12.9	11.7	0.05	0.00	2.30
Pyrene		6.19	4.66	11.3	22.4	21.8	24.4	24.8	20.7	0.83	5.70	10.4
Benz(a)anthracene		0.64	0.52	1.05	1.33	0.97	1.82	2.05	1.92	0.11	0.05	1.28
Benzo(b)fluoranthene		0.85	0.83	1.19	1.33	1.45	1.31	1.93	1.36	0.22	2.69	2.84
Benzo(k)fluoranthene		0.13	0.12	0.21	0.21	0.22	0.25	0.26	0.29	0.43	0.29	0.30
Benzo(e)pyrene		2.70	2.27	2.72	3.27	3.18	3.21	3.28	2.71	8.21	9.47	7.40
Benzo(a)pyrene		1.28	0.97	1.91	2.50	2.62	2.57	2.52	2.06	0.81	1.58	1.57
Perylene		0.53	0.33	0.70	1.32	1.04	1.04	1.31	1.18	0.32	0.54	1.26
Indeno(1,2,3-cd)pyrene		0.31	0.23	0.25	0.10	0.20	0.24	0.00	0.15	0.00	0.00	0.00
Dibenzo(ah)anthracene		0.61	0.42	0.53	0.60	0.60	0.51	0.56	0.51	0.43	0.77	0.49
Benzo(ghi)perylene		1.32	1.15	1.17	1.31	1.30	1.31	1.33	0.94	1.30	2.71	1.91
Total EPA priority PAHs (µg/g)		**20.8**	**16.9**	**42.3**	**35.4**	**33.6**	**38.6**	**288**	**231**	**11.2**	**23.9**	**82.3**
Total PAHs (µg/g sample)		**3138**	**2512**	**8612**	**3215**	**2069**	**5699**	**33967**	**31172**	**309**	**421**	**10671**
Mean (µg/g sample)			**4754**			**3661**			**32570**			
Diagnostic Ratios												
2-m-N:1-m-N		1.39	1.14	1.40	2.30	2.00	0.94	1.33	1.27	1.31	1.58	1.37
4-:2-/3-:1-m-DBT		1.00:0.72:0.25	1.00:0.76:0.27	1.00:0.85:0.23	1.00:0.10:0.24	1.00:0.11:0.48	1.00:0.61:0.27	1.00:1.02:0.21	1.00:1.01:0.21	1.00:0.492.52	1.00:0.19:7.77	1.00:0.73:0.27
(3-+2-)/(4-/9-+1-m-phen)		1.51	1.72	1.72	0.43	0.21	1.35	2.27	2.29	1.67	6.00	1.13
(C2D/C2P):(C3D/C3P)		0.54:0.79	0.54:0.82	0.52:0.84	0.40:0.55	0.43:0.64	0.46:0.52	0.51:0.81	0.51:0.80	0.58:0.69	0.16:0.37	0.84:0.83
C0N:C1N:C2N:C3N:C4N		0.00:0.03:0.13:0.37:1.00	0.00:0.01:0.16:0.49:1.00	0.00:0.00:0.06:0.52:1.0	0.00:0.00:0.00:0.01:1.0	0.00:0.00:0.00:0.01:1.0	0.00:0.00:0.05:0.27:1.0	0.00:0.07:0.74:1.42:1.00	0.00:0.08:0.70:1.38:1.00	0.03:0.19:0.36:0.36:1.00	0.04:0.08:0.16:0.16:1.00	0.04:0.57:1.43:1.85:1.00
Naphs:Phens:DBTs:Fluors:Chrys		1.07:1.00:0.51:0.69:0.06	1.01:1.00:0.49:0.61:0.05	0.95:1.00:0.45:0.63:0.03	1.27:1.00:0.43:0.54:0.11	0.82:1.00:0.45:0.52:0.13	1.54:1.00:0.40:0.72:0.06	1.95:1.00:0.38:0.59:0.01	1.92:1.00:0.37:0.59:0.01	1.06:1.00:0.46:0.47:1.01	0.47:1.00:0.09:0.17:2.38	2.81:1.00:0.69:0.52:0.12

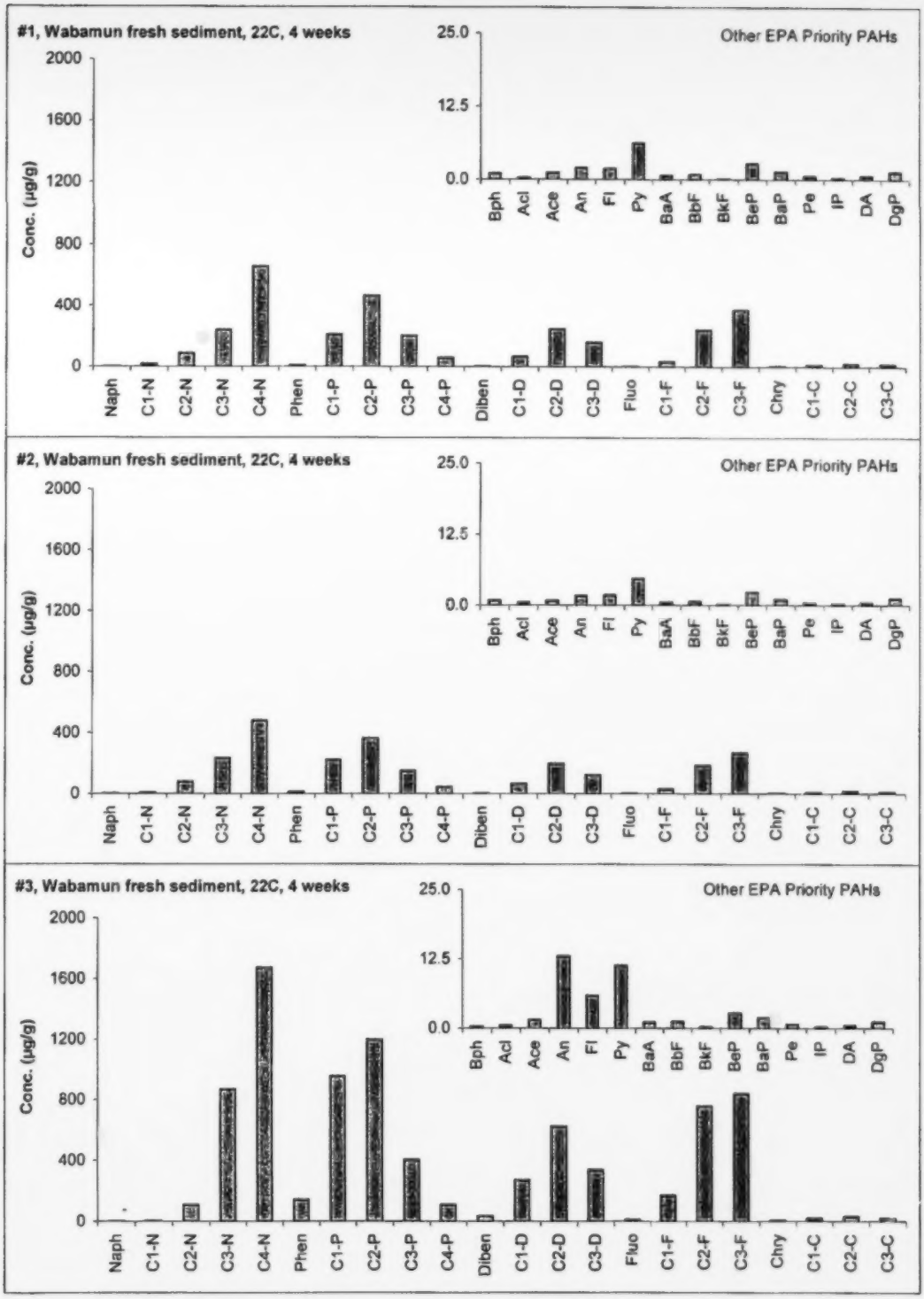
#1, Wabamun fresh sediment, 22C, 4 weeks
Other EPA Priority PAHs
Conc. (µg/g)
2000
1600
1200
800
400
0
25.0
12.5
0.0
Naph C1-N C2-N C3-N C4-N Phen C1-P C2-P C3-P C4-P Diben C1-D C2-D C3-D Fluo C1-F C2-F C3-F Chry C1-C C2-C C3-C
Bph Acl Ace An Fl Py BaA BbF BkF BeP BaP Pe IP DA DgP
#2, Wabamun fresh sediment, 22C, 4 weeks
Other EPA Priority PAHs
Conc. (µg/g)
#3, Wabamun fresh sediment, 22C, 4 weeks
Other EPA Priority PAHs
Conc. (µg/g)

Figure 6 PAHs distributin

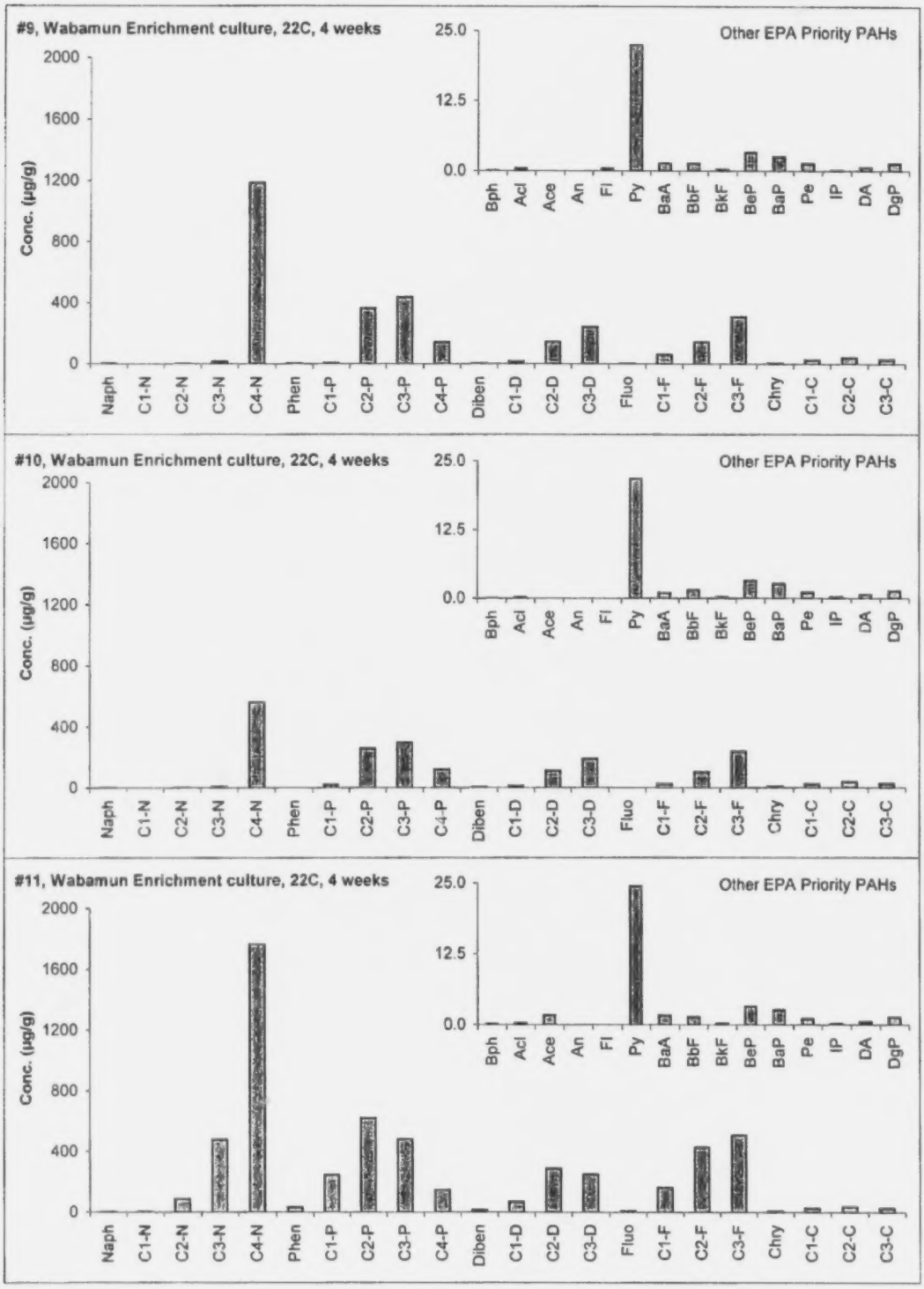
#9, Wabamun Enrichment culture, 22C, 4 weeks
Other EPA Priority PAHs
Conc. (µg/g)
2000
1600
1200
800
400
0
25.0
12.5
0.0
Naph C1-N C2-N C3-N C4-N Phen C1-P C2-P C3-P C4-P Diben C1-D C2-D C3-D Fluo C1-F C2-F C3-F Chry C1-C C2-C C3-C
Bph Acl Ace An Fl Py BaA BbF BkF BeP BaP Pe IP DA DgP
#10, Wabamun Enrichment culture, 22C, 4 weeks
Other EPA Priority PAHs
Conc. (µg/g)
#11, Wabamun Enrichment culture, 22C, 4 weeks
Other EPA Priority PAHs
Conc. (µg/g)

Figure 6 PAHs distributin

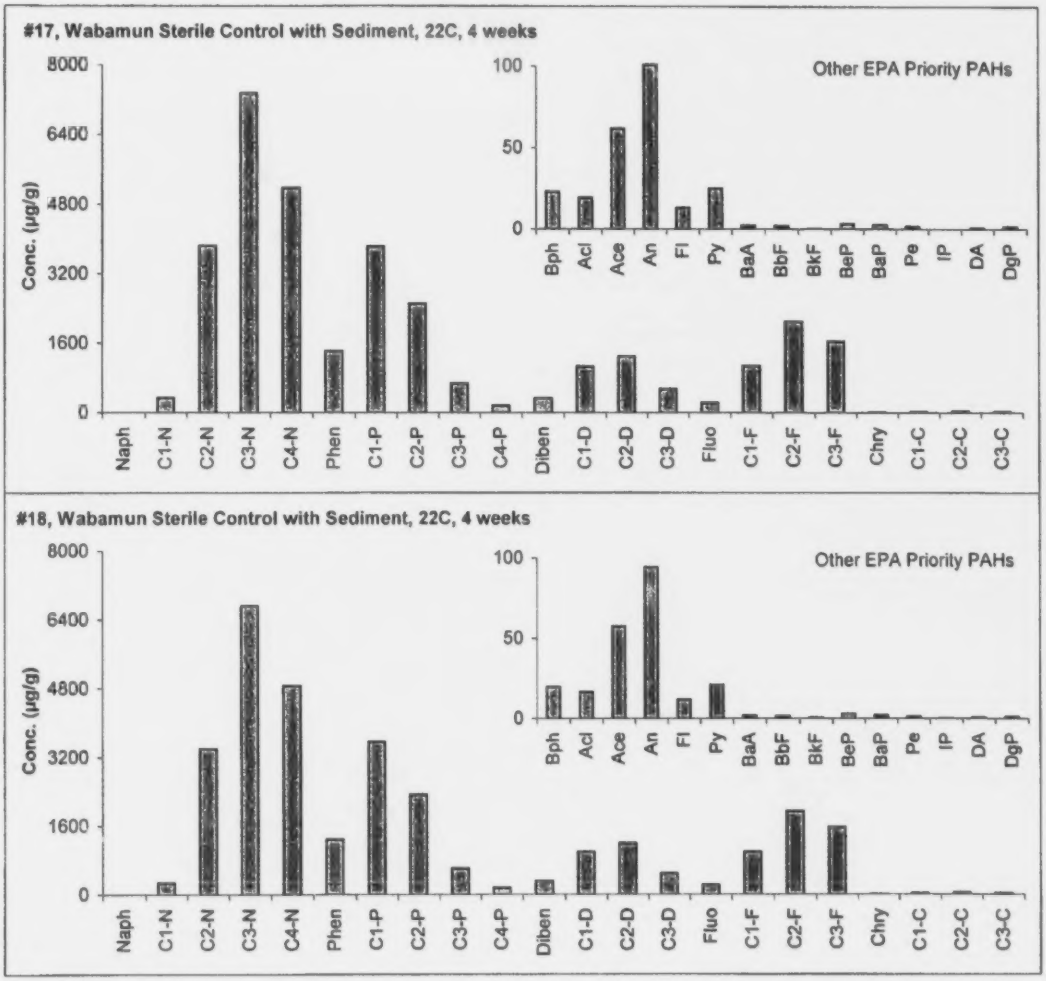

Figure 6 PAHs distributin

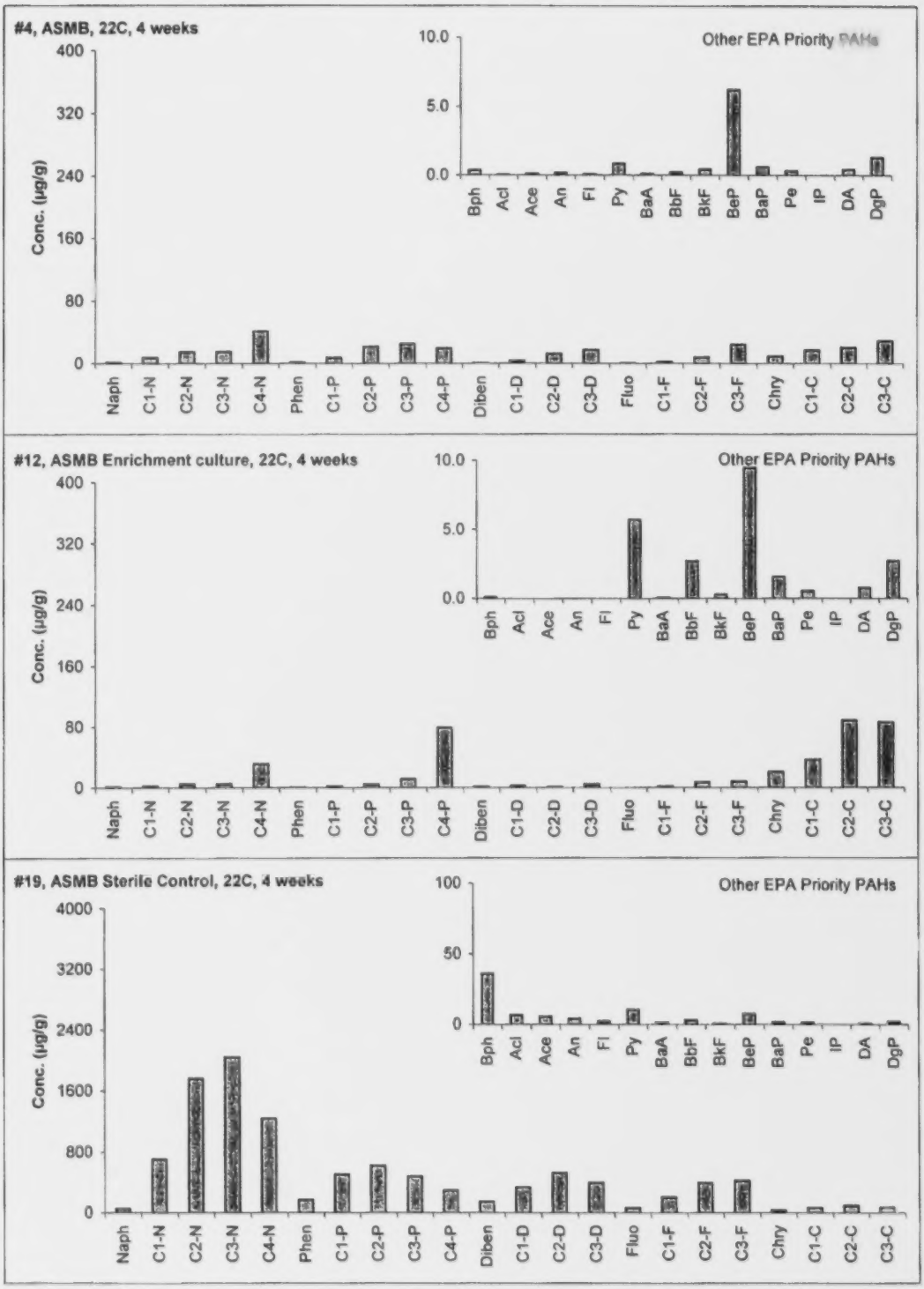
#4, ASMB, 22C, 4 weeks
Conc. (µg/g)
400
320
240
160
80
0
Naph C1-N C2-N C3-N C4-N Phen C1-P C2-P C3-P C4-P Diben C1-D C2-D C3-D Fluo C1-F C2-F C3-F Chry C1-C C2-C C3-C
Other EPA Priority PAHs
10.0
5.0
0.0
Bph Acl Ace An Fl Py BaA BbF BkF BeP BaP Pe IP DA DgP
#12, ASMB Enrichment culture, 22C, 4 weeks
Conc. (µg/g)
400
320
240
160
80
0
Naph C1-N C2-N C3-N C4-N Phen C1-P C2-P C3-P C4-P Diben C1-D C2-D C3-D Fluo C1-F C2-F C3-F Chry C1-C C2-C C3-C
Other EPA Priority PAHs
10.0
5.0
0.0
Bph Acl Ace An Fl Py BaA BbF BkF BeP BaP Pe IP DA DgP
#19, ASMB Sterile Control, 22C, 4 weeks
Conc. (µg/g)
4000
3200
2400
1600
800
0
Naph C1-N C2-N C3-N C4-N Phen C1-P C2-P C3-P C4-P Diben C1-D C2-D C3-D Fluo C1-F C2-F C3-F Chry C1-C C2-C C3-C
Other EPA Priority PAHs
100
50
0
Bph Acl Ace An Fl Py BaA BbF BkF BeP BaP Pe IP DA DgP

Figure 6 PAHs distributin

Table 5. Chemical composition changes of hydrocarbon groupings

Sample	Wabamun						ASMB		
	Fresh sediment, 22C, 4 weeks		Enrichment culture, 22C, 4 weeks		Sterile Control with Sediment, 22C, 4 weeks		ASMB, 22C, 4 weeks	ASMB Enrichment culture, 22C, 4 weeks	ASMB Sterile Control, 22C, 4 weeks
	n=3	RSD (%)	n=3	RSD (%)	n=2	RSD (%)	n=1	n=1	n=1
Hydrocarbons									
TPH (mg/g sample)	114	21.4	131	9.8	239	4.0	152	196	415
TSH (mg/g sample)	79.0	20.4	80.7	8.0	133	4.2	109	114	274
TAH (mg/g sample)	34.5	38.5	50.3	16.7	106	3.8	43.3	81.6	141
n-Alkane (mg/g sample)	0.33	30.0	0.13	45.6	8.18	6.6	0.00	0.00	46.8
Total 5-PAH hmologues (µg/g sample)	4727	70.7	3625	51.1	32327	6.1	298	397	10588
Total other 15 USEPA priority PAHs (µg/g sample)	26.7	51.3	35.8	7.1	243	7.1	11.2	23.9	82.3
Hydrocarbon lost									
TPH (%)	52.3		45.2				63.4	52.8	
TSH (%)	40.6		39.3				60.2	58.4	
TAH (%)	67.5		52.5				69.3	42.1	
n-Alkane (%)	96.0		98.4				100	100	
Total 5-PAH hmologues (%)	85.4		88.8				97.2	96.3	
Total other 15 USEPA priority PAHs (%)	89.0		85.3				86.4	71.0	

Memorandum

Report: 2006-05

Date: May 26, 2006

To: Gary Sergy
Head, Prarie and Northern Section

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Subject: **Characterization of the Biodegraded Wabamun Spill Oil Samples (2nd Batch) Incubated with Lake Wabamun Sediment or Wabamun Enrichment Culture at 4 ^{0}C for 8 weeks**

This Memorandum summarizes the characterization results for the biodegradaed Wabamun spill oil samples with Lake Wabamun Sediment or Wabamun Enrichment Culture at 4 ^{0}C for 8 weeks. For the detailed study background, biodegadation method, sample preparation, instrumentation and GC programs used, please refer ***Report 2006-04***.

Table 1 Wabamun oil degradation experiment
(4 ^{0}C, 8 weeks; set up on January 25, 2006)

Sample type	Sample (ESTD #)	Oil type	Weigh oil added (g)	Wt. Vial (g)	Wt. Vial + oil (g)	Wt. Oil recovered* (g)	% recovered
Wab Fresh Sediments	849	Wab 1	0.392				
	850	Wab 1	0.392				
	851	Wab 1	0.392				
	852	ASMB	0.399	4.8714	5.0093	0.1379	35

Wab Enrichment Culture	853 854 855 856	Wab 1 Wab 1 Wab 1 ASMB	0.392 0.392 0.392 0.4125	4.8475	5.0894	0.2419	59
Sterile Control with Sediments	857 858 859	Wab 1 Wab 1 ASMB	0.392 0.392 0.4095	4.9032	5.1286	0.2244	55

Characterization Results and Discussion

(1) Determination of Hydrocarbon Groups and n-Alkane Distribution

1. Figures 1, 2 and 3 show the GC/FID chromatograms of F1, F2 and F3 for total saturates, total aromatics, and total petroleum hydrocarbon (TPH) analyses, respectively. Comparison of the GC chromatograms immediately reveals that biodegradation had been occurred during 8 weeks inoculation.

Table 2 summarizes the hydrocarbon group quantitation results of the samples. Table 3 summarizes the n-alkane values in biodegraded samples. Figure 4 depicts graphically the n-alkane distribution in biodegraded samples. *Note that all calculations were based on oil weight added, not on oil weight recovered*

(2) Determination of Target Alkylated PAH Homologues and Their Diagnostic Ratios

Figure 5 shows the total ion GC/MS chromatograms (in SIM mode) of the samples for analyses of target alkylated PAH homologues. Table 4 summarizes quantitation results of 5 petroleum-characteristic alkylated PAH homologous series (alkylated naphthalene, phenanthrene, dibenzothiophene, fluorene, and chrysene series) and other EPA priority PAHs. For comparison, diagnostic ratios of some target PAH groups and paired isomers are also presented in Table 4. Figure 6 graphically depicts the distribution of these target PAH compounds.

(3) Characterization of Terpane and Sterane Biomarkers

The biomarker characterization results clearly demonstrated there was no observable sigh of alteration of biomarker terpane and steranes (detailed results are not shown here).

(4) Percentage Loss of Hydrocarbon Groups

Table 5 summarizes the quantitative composition changes and percentage

loss of hydrocarbon groups in 11 tested biodegradation oil samples.

(1) Biodegradation of the Wabamun spill oil by microbial is clearly demonstrated.

(2) In comparison to the 1st batch biodegraded samples (22 ^{0}C, 4 weeks), less oil hydrocarbon degradation of the 2nd batch samples is apparent, clearly indicating effects of temperature on the biodegradation degree of the Wabamun oil samples.

(3) It is noted that in comparison to the biodegradation at 22 ^{0}C for 28 days, the Wabamun fresh sediment samples did not show significant differences in total hydrocarbon degradation from the Wabamun enrichment culture samples under the current biodegradation conditions (4 ^{0}C, 8 weeks).

Zhendi Wang

No.: CY-057-2006

Fingerprinting Analysis of Oil Samples

Project: Biodegradated Oil Samples from University of Alberta [2006] (Batch II)

Biodegradated Oil Samples from University of Alberta [2006]		Weight	Volume	PAH	TPH, n-Alkane and Biomarker
ESTD code		(g)	(mL)	Oil in F1 or F2 (mg)	Oil in F1 or F2 (mg)
2006/03/30-0849	#5, Wabamun fresh sediment, 4C, 8 weeks	0.3920	5.0	15.680	15.360
2006/03/30-0850	#6, Wabamun fresh sediment, 4C, 8 weeks	0.3920	5.0	15.680	15.360
2006/03/30-0851	#7, Wabamun fresh sediment, 4C, 8 weeks	0.3920	5.0	15.680	15.360
2006/03/30-0852	#8, ASMB, 4C, 8 weeks	**0.3990**	5.0	**15.960**	**15.634**
2006/03/30-0853	#13, Wabamun Enrichment culture, 4C, 8 weeks	0.3920	5.0	15.680	15.360
2006/03/30-0854	#14, Wabamun Enrichment culture, 4C, 8 weeks	0.3920	5.0	15.680	15.360
2006/03/30-0855	#15, Wabamun Enrichment culture, 4C, 8 weeks	0.3920	5.0	15.680	15.360
2006/03/30-0856	#16, ASMB Enrichment culture, 4C, 8 weeks	**0.4125**	5.0	**16.500**	**16.163**
2006/03/30-0857	#20, Wabamun Sterile Control with Sediment, 4C, 8 weeks	0.3920	5.0	15.680	15.360
2006/03/30-0858	#21, Wabamun Sterile Control with Sediment, 4C, 8 weeks	0.3920	5.0	15.680	15.360
2006/03/30-0859	#22, ASMB Sterile Control, 4C, 8 weeks	**0.4095**	5.0	**16.380**	**16.046**
2006/03/02-0773	#1, Wabamun fresh sediment, 22C, 4 weeks	0.3731	5.0	14.924	11.878
2006/03/02-0780	#12, ASMB Enrichment culture, 22C, 4 weeks	0.1956	5.0	7.824	7.664
Reference oil	Ref. oil: Prudhoe Bay 13.1%	0.8008	10.0	16.016	

* 0.016g d10-phenanthrene (0.4 mg in 25 mL) added in 0.4 g oil as surrogate, 0.64 ug in 200ul solution for analysis

Version: 1

ESTD/ETC
Environment Canada

14-Jul-06

Table 2. GC-TPH analysis results

Sample	Wabamun fresh sediment, 4C, 8 weeks					Wabamun Enrichment culture, 4C, 8 weeks					Wabamun Sterile Control with Sediment, 4C, 8 weeks				ASMB, 4C, 8 weeks	ASMB Enrichment culture, 4C, 8 weeks	ASMB sterile Control 4C, 8 weeks
ESTD Log No.	2006/03/30 0849	2006/03/30 0850	2006/03/30 0851	Mean	RSD	2006/03/30 0853	2006/03/30 0854	2006/03/30 0855	Mean	RSD	2006/03/30 0857	2006/03/30 0858	Mean	RSD	2006/03/30-0852	2006/03/30-0856	2006/03/30-0859
Client Sample No.	#5	#6	#7		(%)	#13	#14	#15		(%)	#20	#21		(%)	#8	$16	#22
TPH (mg/g sample)	167	156	173	**172**	9.1	149	169	165	**161**	6.8	235	233	**234**	0.6	133	250	315
TSH (mg/g sample)	129	113	115	**119**	7.4	99.0	114	110	**108**	7.2	132	133	**133**	0.2	100	175	230
TAH (mg/g sample)	57.8	43.0	57.7	**52.9**	16.1	49.6	55.4	55.0	**53.3**	6.1	103	101	**102**	1.7	33.0	75.0	85.0
TSH/TPH (%)	69.1	72.4	66.6	**69.4**	4.2	66.6	67.3	66.7	**66.9**	0.5	56.3	56.9	**56.6**	0.9	75.2	70.0	73.0
TAH/TPH (%)	30.9	27.6	33.4	**30.6**	9.5	33.4	32.7	40.4	**35.5**	12.0	43.7	43.1	**43.4**	1.1	24.8	30.0	27.0
Resolved peaks/TPH (%)	12.4	8.2	13.1	**11.2**	23.6	14.1	16.77	15.8	**15.5**	8.8	24.4	25.5	**24.9**	3.1	5.9	6.4	25.6

* TSH: total saturated hydrocarbons; TAH: total aromatic hydrocarbons.

Table 3. n-Alkane analysis results

Sample	Wabamum fresh sediment, 4C, 8 weeks			Wabamum Enrichment Culture, 4C, 8 weeks			Wabamun Sterile Control with Sediment, 4C, 8 weeks		ASMB, 4C, 8 weeks	ASMB Enrichment culture, 4C, 8 weeks	ASMB Sterile Control, 4C, 8 weeks
ESTD Sample No.	2006/03/30-0849	2006/03/30-0850	2006/03/30-0851	2006/03/30-0853	2006/03/30-0854	2006/03/30-0855	2006/03/30-0857	2006/03/30-0858	2006/03/30-0852	2006/03/30-0856	2006/03/30-0859
Client sample information	#5	#6	#7	#13	#14	#15	#20	#21	#8	#16	#22
n-Alkane (mg/g sample)											
n-C9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
n-C10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05
n-C11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.61
n-C12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.54
n-C13	0.01	0.00	0.01	0.00	0.00	0.01	0.05	0.03	0.00	0.00	2.44
n-C14	0.09	0.00	0.03	0.00	0.00	0.04	0.30	0.23	0.00	0.00	2.82
n-C15	0.22	0.00	0.08	0.00	0.00	0.10	0.72	0.63	0.01	0.00	3.08
n-C16	0.27	0.00	0.10	0.00	0.00	0.13	0.87	0.85	0.01	0.00	2.87
n-C17	0.30	0.00	0.13	0.00	0.00	0.15	0.97	0.98	0.04	0.03	2.74
Pri	0.20	0.00	0.20	0.00	0.00	0.11	0.38	0.38	0.07	0.03	2.06
n-C18	0.28	0.00	0.13	0.00	0.00	0.14	0.91	0.90	0.05	0.04	2.48
Phy	0.24	0.00	0.19	0.00	0.00	0.11	0.33	0.36	0.10	0.04	1.62
n-C19	0.30	0.00	0.13	0.01	0.01	0.15	0.89	0.92	0.08	0.08	2.37
n-C20	0.30	0.00	0.14	0.01	0.01	0.15	0.94	0.97	0.05	0.05	2.08
n-C21	0.26	0.02	0.12	0.02	0.02	0.14	0.76	0.80	0.05	0.06	1.90
n-C22	0.17	0.01	0.09	0.01	0.01	0.09	0.48	0.50	0.05	0.05	1.68
n-C23	0.10	0.02	0.06	0.01	0.01	0.06	0.27	0.29	0.05	0.05	1.47
n-C24	0.06	0.01	0.04	0.01	0.01	0.03	0.13	0.15	0.06	0.05	1.39
n-C25	0.05	0.02	0.04	0.01	0.01	0.02	0.09	0.11	0.08	0.05	1.41
n-C26	0.01	0.01	0.01	0.01	0.00	0.01	0.03	0.04	0.06	0.05	1.20
n-C27	0.05	0.04	0.04	0.01	0.00	0.01	0.08	0.13	0.08	0.04	1.06
n-C28	0.01	0.02	0.02	0.01	0.01	0.01	0.02	0.03	0.06	0.04	0.90
n-C29	0.02	0.04	0.03	0.02	0.01	0.02	0.03	0.06	0.06	0.05	0.82
n-C30	0.01	0.04	0.02	0.02	0.01	0.02	0.01	0.03	0.06	0.05	0.68
n-C31	0.01	0.03	0.02	0.01	0.01	0.02	0.02	0.03	0.05	0.04	0.57
n-C32	0.01	0.03	0.02	0.01	0.01	0.02	0.01	0.02	0.05	0.04	0.43
n-C33	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.05	0.05	0.36
n-C34	0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.02	0.05	0.04	0.38
n-C35	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.06	0.04	0.35
n-C36	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.03	0.15
n-C37	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.02	0.02	0.10
n-C38	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.01	0.02	0.08
n-C39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.05
n-C40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.04
Total	**3.12**	**0.42**	**1.72**	**0.21**	**0.15**	**1.58**	**8.32**	**8.50**	**1.33**	**1.08**	**41.8**
Mean		**1.75**			**0.65**			**8.41**			
Diagnostic indexes											
n-C17/Pristane	1.08	NA	0.67	NA	NA	1.34	2.54	2.56	0.52	1.06	1.32
n-C18/Phytane	1.17	NA	0.67	NA	NA	1.28	2.79	2.50	0.51	0.99	1.53
Pr/Ph	1.15	NA	1.01	NA	NA	0.97	1.17	1.06	0.71	0.63	1.29
Odd alkanes	1.35	0.23	0.69	0.11	0.08	0.69	3.90	4.01	0.61	0.52	19.3
Even alkenes	1.25	0.19	0.64	0.11	0.07	0.67	3.71	3.74	0.54	0.50	18.8
CPI (odd/even)	1.08	1.18	1.06	0.99	1.13	1.02	1.05	1.07	1.14	1.04	1.03

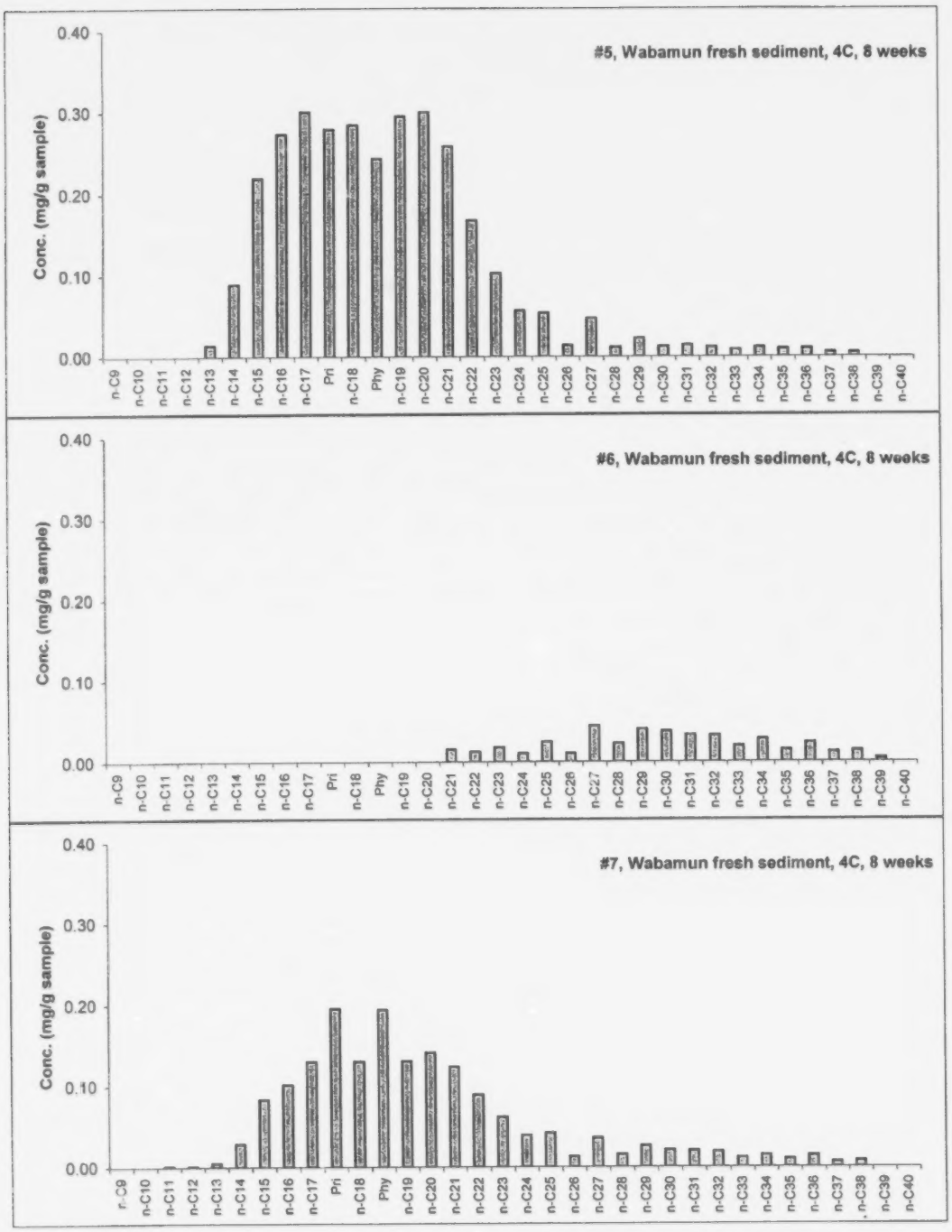

Figure 4 n-Alkane distribution

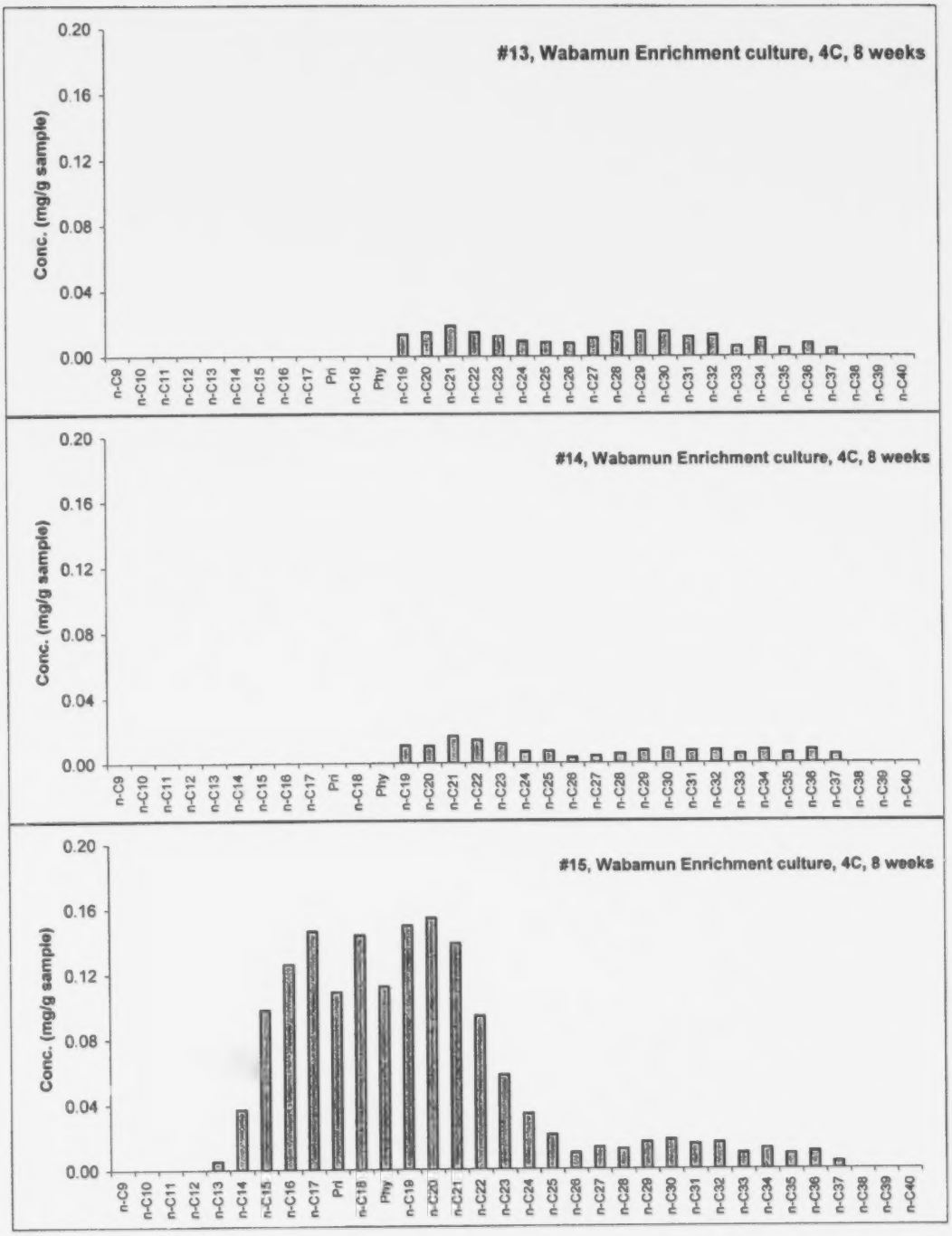
#13, Wabamun Enrichment culture, 4C, 8 weeks
#14, Wabamun Enrichment culture, 4C, 8 weeks
#15, Wabamun Enrichment culture, 4C, 8 weeks
Conc. (mg/g sample)
0.20
0.16
0.12
0.08
0.04
0.00
n-C9 n-C10 n-C11 n-C12 n-C13 n-C14 n-C15 n-C16 n-C17 Pri n-C18 Phy n-C19 n-C20 n-C21 n-C22 n-C23 n-C24 n-C25 n-C26 n-C27 n-C28 n-C29 n-C30 n-C31 n-C32 n-C33 n-C34 n-C35 n-C36 n-C37 n-C38 n-C39 n-C40

Figure 4 n-Alkane distribution

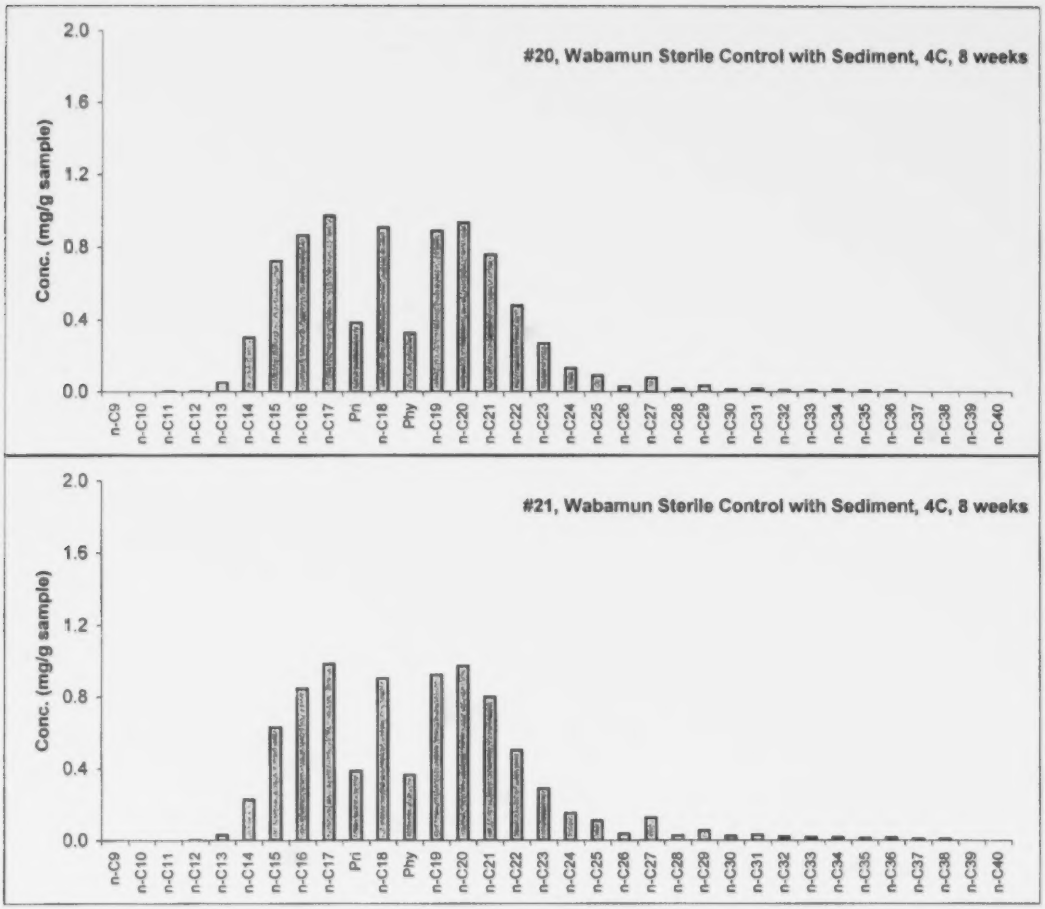
#20, Wabamun Sterile Control with Sediment, 4C, 8 weeks
Conc. (mg/g sample)
2.0
1.6
1.2
0.8
0.4
0.0
n-C9 n-C10 n-C11 n-C12 n-C13 n-C14 n-C15 n-C16 n-C17 Pri n-C18 Phy n-C19 n-C20 n-C21 n-C22 n-C23 n-C24 n-C25 n-C26 n-C27 n-C28 n-C29 n-C30 n-C31 n-C32 n-C33 n-C34 n-C35 n-C36 n-C37 n-C38 n-C39 n-C40
#21, Wabamun Sterile Control with Sediment, 4C, 8 weeks
Conc. (mg/g sample)
2.0
1.6
1.2
0.8
0.4
0.0
n-C9 n-C10 n-C11 n-C12 n-C13 n-C14 n-C15 n-C16 n-C17 Pri n-C18 Phy n-C19 n-C20 n-C21 n-C22 n-C23 n-C24 n-C25 n-C26 n-C27 n-C28 n-C29 n-C30 n-C31 n-C32 n-C33 n-C34 n-C35 n-C36 n-C37 n-C38 n-C39 n-C40

Figure 4 n-Alkane distribution

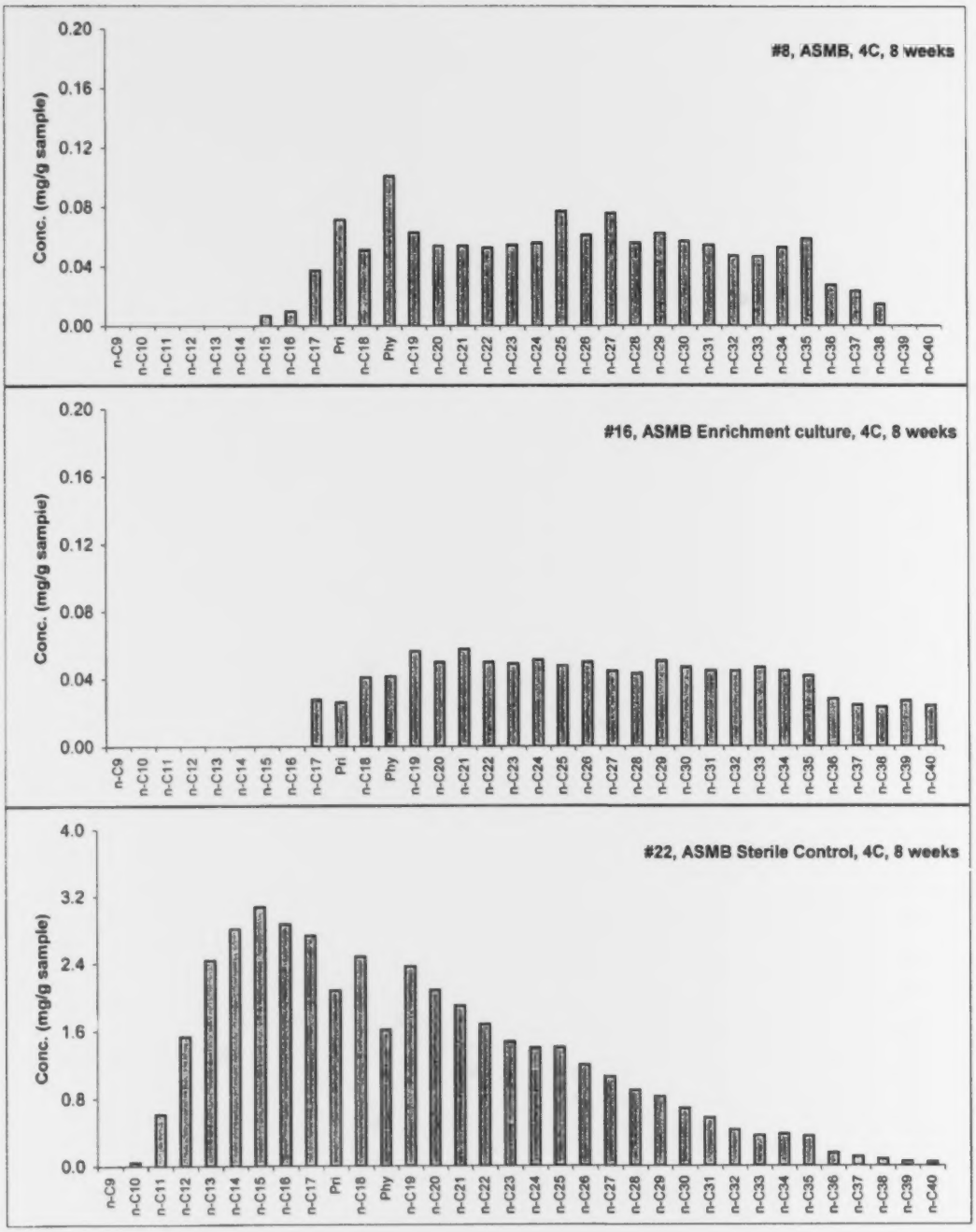
#8, ASMB, 4C, 8 weeks
#16, ASMB Enrichment culture, 4C, 8 weeks
#22, ASMB Sterile Control, 4C, 8 weeks
Conc. (mg/g sample)
0.20
0.16
0.12
0.08
0.04
0.00
4.0
3.2
2.4
1.6
0.8
0.0
n-C9 n-C10 n-C11 n-C12 n-C13 n-C14 n-C15 n-C16 n-C17 Pri n-C18 Phy n-C19 n-C20 n-C21 n-C22 n-C23 n-C24 n-C25 n-C26 n-C27 n-C28 n-C29 n-C30 n-C31 n-C32 n-C33 n-C34 n-C35 n-C36 n-C37 n-C38 n-C39 n-C40

Figure 4 n-Alkane distribution

Table 4 PAHs analysis results

Sample		Wabamun fresh sediment, 4C, 8 weeks			Wabamun Enrichment culture, 4C, 8 weeks			Wabamun Sterile Control with Sediment, 4C, 8 weeks		ASMB, 4C, 8 weeks	ASMB Enrichment culture, 4C, 8	ASMB Sterile Control, 4C, 8
ESTD Log No.		2006/30/30-0849	2006/30/30-0850	2006/30/30-0851	2006/30/30-0853	2006/30/30-0854	2006/30/30-0855	2006/30/30-0857	2006/30/30-0858	2006/03/30-0852	2006/03/30-0856	2006/03/30-0859
Client Sample No.		#5	#6	#7	#13	#14	#15	#20	#21	#8	#16	#21
Alkylated PAHs (µg/g sample)												
Naphthalene	C0-N	1.04	1.00	0.98	0.87	0.86	0.93	1.17	1.19	1.00	0.78	42.5
	C1-N	4.07	2.63	24.6	1.30	1.30	43.4	302	127	1.36	0.00	493
	C2-N	168	29.5	549	25.3	74.2	778	3649	2452	4.63	0.00	1302
	C3-N	1244	344	1862	599	1796	2135	6770	5588	11.8	8.31	1496
	C4-N	2199	1124	2459	2528	3813	2491	4924	4175	52.4	434	916
	Sum	**3616**	**1501**	**4896**	**3155**	**5685**	**5448**	**15645**	**12353**	**71.2**	**443.0**	**4250**
Phenanthrene	C0-P	147	20.0	252	32.3	18.5	260	1382	1322	1.28	1.29	119
	C1-P	1273	340	1304	498	764	1033	3702	3719	17.0	6.15	365
	C2-P	1505	829	1421	1152	1785	1195	2393	2483	52.3	212	449
	C3-P	555	414	499	568	621	113	608	665	65.0	478	374
	C4-P	160.0	135.9	139	156	150	157	153	165	37.0	314	240
	Sum	**3640**	**1739**	**3616**	**2437**	**3338**	**2758**	**8239**	**8354**	**173**	**1009**	**1547**
Dibenzothiophene	C0-D	33.9	7.27	53.5	14.2	10.4	65.0	314	298	0.43	1.04	95.8
	C1-D	342	98.7	335	159	300	292	978	978	7.50	0.20	212
	C2-D	763	412	679	623	1009	617	1180	1185	30.0	195	346
	C3-D	459	329	376	384	481	397	489	493	39.0	302	280
	Sum	**1597**	**845**	**1444**	**1180**	**1801**	**1371**	**2961**	**2954**	**77.0**	**498**	**914**
Fluorene	C0-F	9.23	2.38	30.2	5.24	5.55	40.0	223	189	0.26	0.67	45.4
	C1-F	209	73.1	287	176	303	315	1026	969	3.62	14.1	145
	C2-F	906	474	989	858	1527	922	2002	1965	25.5	49.4	277
	C3-F	1134	725	1065	960	1479	998	1606	1627	54.1	171	323
	Sum	**2258**	**1274**	**2352**	**1999**	**3315**	**2274**	**4857**	**4750**	**83.5**	**235**	**790**
Chrysene	C0-C	7.61	5.93	7.10	7.38	7.45	6.98	7.62	7.95	9.24	30.3	21.2
	C1-C	27.5	24.7	23.3	26.1	25.4	26.6	25.2	24.2	16.3	74.0	44.2
	C2-C	42.6	38.3	33.7	37.7	37.4	36.0	34.1	33.8	28.2	105	66.0
	C3-C	32.9	28.3	27.7	28.5	25.8	24.9	24.0	25.7	36.5	88.1	58.2
	Sum	**110.6**	**97.2**	**91.8**	**100**	**96.0**	**94.4**	**90.9**	**91.6**	**90.2**	**297**	**190**
Total alkylated PAHS		**11221**	**5457**	**12399**	**8871**	**14236**	**11945**	**31793**	**28503**	**494**	**2482**	**7690**
Other Priority PAHs (µg/g sample												
Biphenyl		0.29	0.00	1.39	0.00	0.00	2.96	20.4	13.0	0.00	0.00	24.4
Acenaphthylene		1.41	0.00	3.05	0.00	0.00	3.55	15.9	12.9	0.00	0.00	4.69
Acenaphthene		1.80	0.00	7.74	0.00	0.00	17.3	61.0	39.4	0.00	0.00	3.05
Anthracene		16.7	2.41	20.2	0.00	0.00	20.7	90.7	80.8	0.00	0.00	2.10
Fluoranthene		5.76	3.37	6.51	5.04	8.43	6.14	11.9	13.0	0.00	2.01	1.79
Pyrene		16.4	12.7	15.7	22.5	23.7	22.2	23.4	22.7	2.09	11.2	6.73
Benz(a)anthracene		1.73	1.28	1.45	1.86	2.01	1.64	2.03	1.76	0.04	1.03	0.50
Benzo(b)fluoranthene		1.68	1.22	1.28	1.23	1.25	1.16	1.14	1.40	0.87	3.68	2.21
Benzo(k)fluoranthene		0.23	0.25	0.35	0.23	0.16	0.27	0.38	0.26	0.00	0.43	0.16
Benzo(e)pyrene		3.70	3.27	3.19	3.04	2.77	2.76	2.81	2.79	4.07	9.20	5.58
Benzo(a)pyrene		3.20	2.78	2.78	2.67	2.50	2.37	2.29	2.29	0.93	1.86	1.24
Perylene		1.44	1.17	1.08	1.00	0.98	0.95	1.19	1.30	0.52	1.43	0.74
Indeno(1,2,3-cd)pyrene		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dibenzo(ah)anthracene		0.79	0.67	0.69	0.66	0.52	0.53	0.52	0.56	0.35	0.61	0.48
Benzo(ghi)perylene		1.89	1.63	1.64	1.47	1.11	1.07	1.16	1.33	1.38	2.76	1.87
Total EPA priority PAHs (µg/g)		**57.0**	**30.7**	**87.0**	**39.7**	**43.6**	**83.7**	**235**	**193**	**10.2**	**34.2**	**55.5**
Total PAHs (µg/g sample)		**11301**	**5487**	**12487**	**8911**	**14279**	**12030**	**32028**	**28696**	**505**	**2516**	**7746**
Mean (µg/g sample)			**9759**			**11740**			**30362**			
Diagnostic Ratios												
2-m-N:1-m-N		1.25	1.73	0.92	1.29	1.38	0.98	1.26	1.19	1.77	0.00	1.36
4-:2-/3-:1-m-DBT		1.00:0.91:0.20	1.00:0.66:0.29	1.00:0.86:0.21	1.00:0.56:0.26	1.00:0.46:0.24	1.00:0.86:0.21	1.00:0.98:0.21	1.00:0.98:0.21	1.00:0.61:0.91	NA	1.00:0.67:0.27
(3-+2-)/(4-/9-+1-m-phen)		2.05	1.14	1.90	0.94	0.67	1.75	2.31	2.30	1.00	0.68	1.08
(C2D/C2P):(C3D/C3P)		0.51:0.83	0.50:0.79	0.48:0.75	0.54:0.64	0.57:0.78	0.52:3.52	0.49:0.80	0.48:0.74	0.57:0.60	0.92:0.63	0.77:0.70
C0N:C1N:C2N:C3N:C4N		0.00:0.00:0.08:0.57:1.00	0.00:0.00:0.03:0.31:1.00	0.00:0.01:0.22:0.76:1.00	0.00:0.00:0.01:0.24:1.00	0.00:0.00:0.02:0.47:1.00	0.00:0.02:0.31:0.86:1.00	0.00:0.08:0.74:1.37:1.00	0.00:0.03:0.59:1.34:1.00	0.02:0.03:0.09:0.23:1.00	0.00:0.00:0.00:0.02:1.00	0.05:0.54:1.42:1.63:1.00
Naphs:Phens:DBTs:Fluors:Chrys		0.99:1.00:0.44:0.62:0.03	0.86:1.00:0.49:0.73:0.06	1.35:1.00:0.40:0.85:0.03	1.29:1.00:0.48:0.82:0.04	1.79:1.00:0.54:0.99:0.03	1.98:1.00:0.50:0.82:0.03	1.90:1.00:0.36:0.59:0.01	1.48:1.00:0.35:0.57:0.03	0.41:1.00:0.45:0.48:0.52	0.44:1.00:0.49:0.23:0.29	2.75:1.00:0.59:0.51:0.12

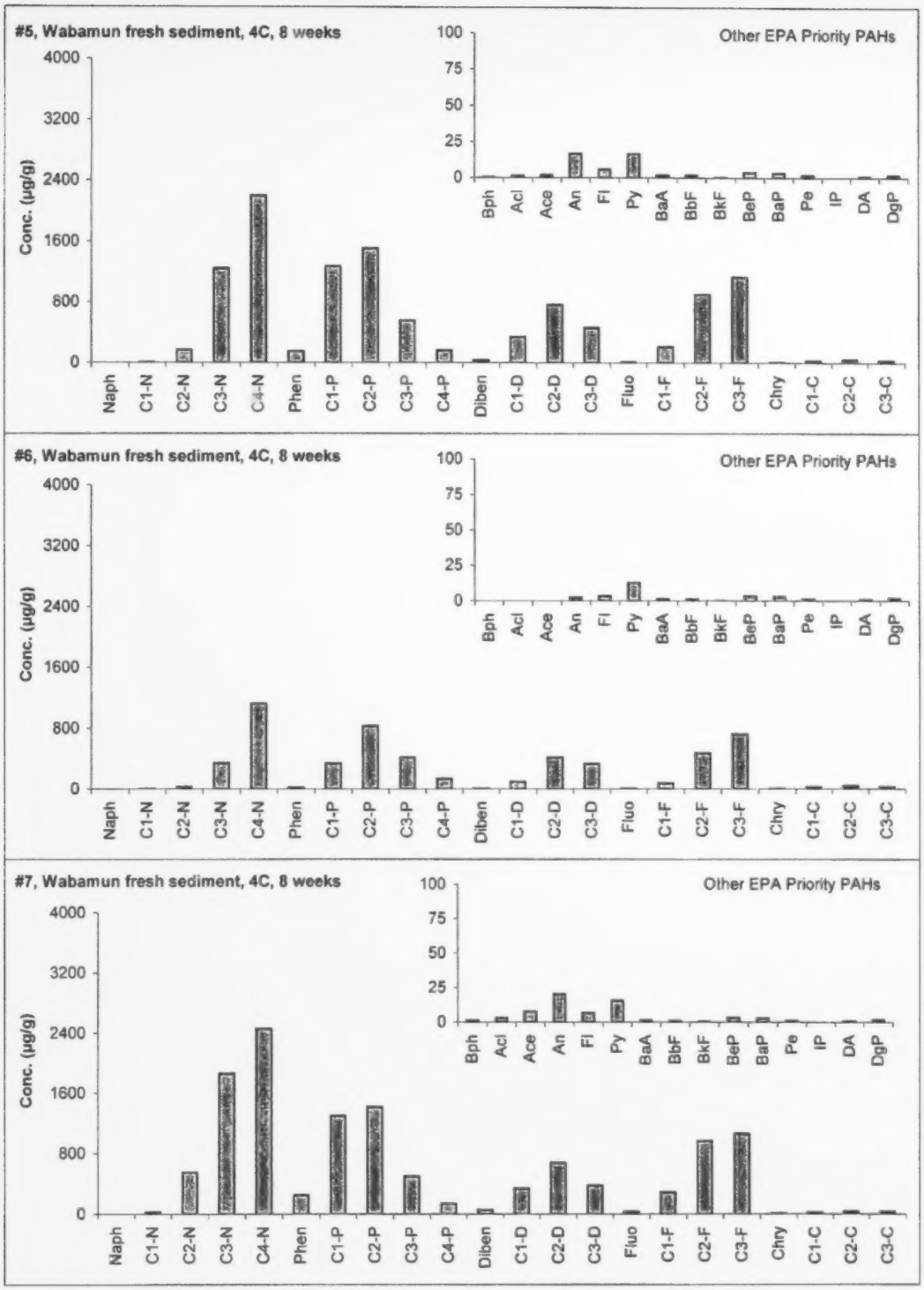
#5, Wabamun fresh sediment, 4C, 8 weeks
Other EPA Priority PAHs
Conc. (µg/g)
4000
3200
2400
1600
800
0
100
75
50
25
0
Naph C1-N C2-N C3-N C4-N Phen C1-P C2-P C3-P C4-P Diben C1-D C2-D C3-D Fluo C1-F C2-F C3-F Chry C1-C C2-C C3-C
Bph Acl Ace An Fl Py BaA BbF BkF BeP BaP Pe IP DA DgP
#6, Wabamun fresh sediment, 4C, 8 weeks
Other EPA Priority PAHs
Conc. (µg/g)
Naph C1-N C2-N C3-N C4-N Phen C1-P C2-P C3-P C4-P Diben C1-D C2-D C3-D Fluo C1-F C2-F C3-F Chry C1-C C2-C C3-C
Bph Acl Ace An Fl Py BaA BbF BkF BeP BaP Pe IP DA DgP
#7, Wabamun fresh sediment, 4C, 8 weeks
Other EPA Priority PAHs
Conc. (µg/g)
Naph C1-N C2-N C3-N C4-N Phen C1-P C2-P C3-P C4-P Diben C1-D C2-D C3-D Fluo C1-F C2-F C3-F Chry C1-C C2-C C3-C
Bph Acl Ace An Fl Py BaA BbF BkF BeP BaP Pe IP DA DgP

Figure 6 PAHs distributin

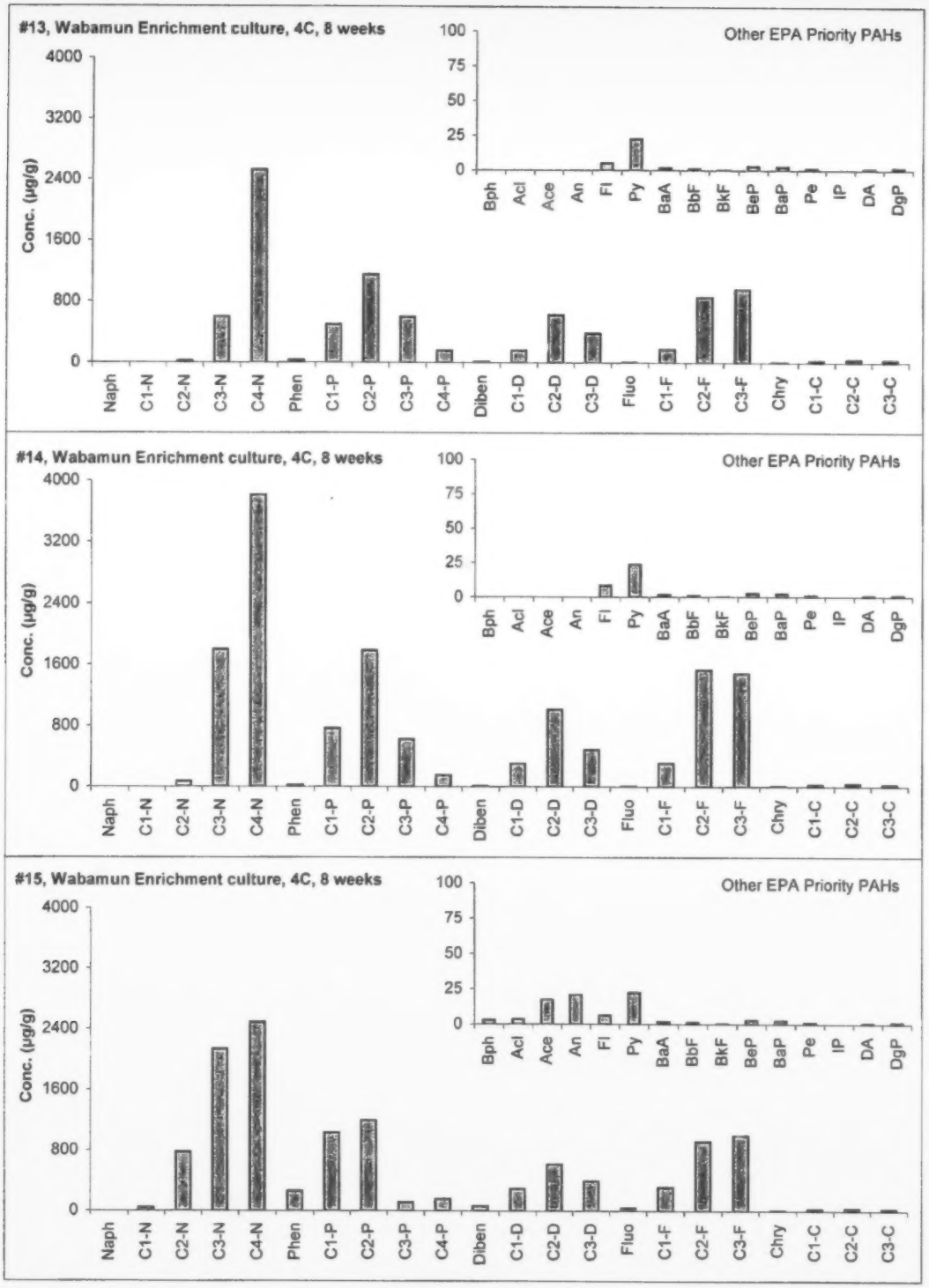

Figure 6 PAHs distributin

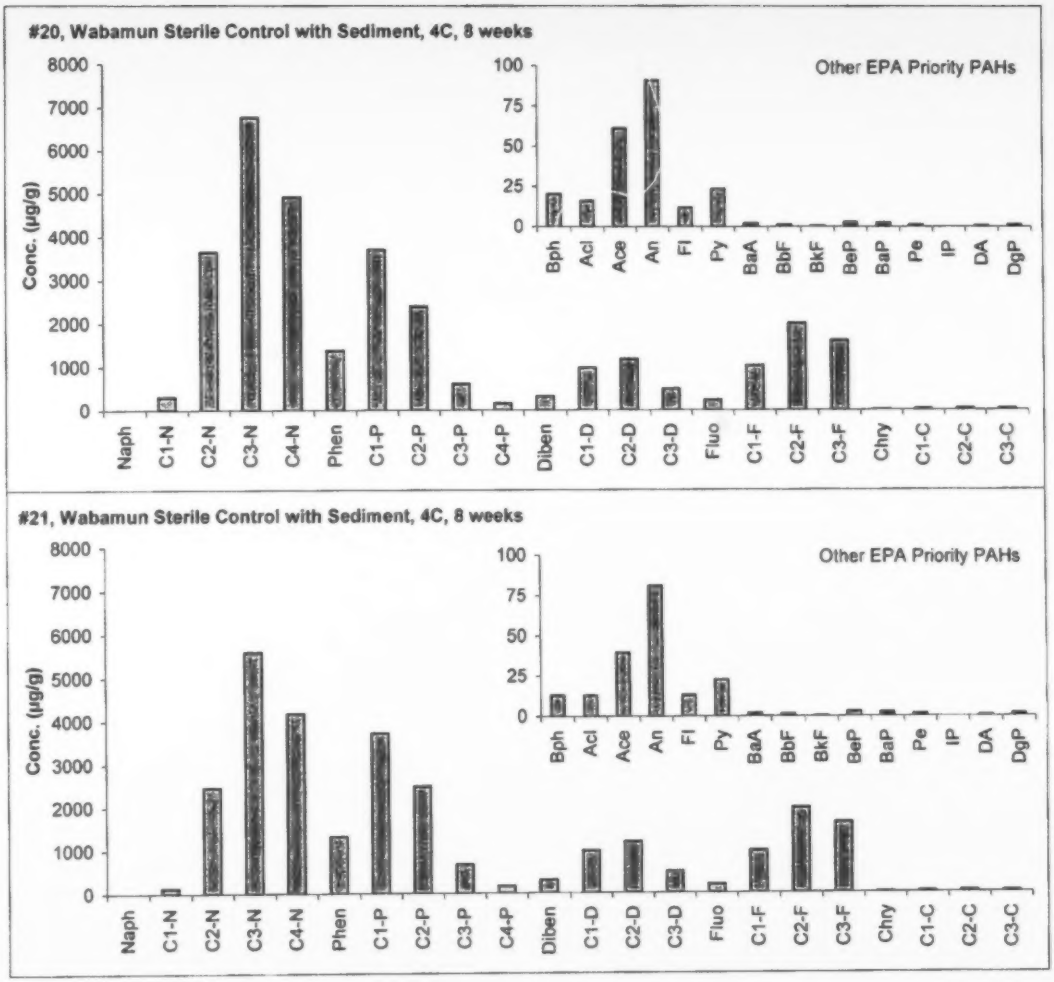
#20, Wabamun Sterile Control with Sediment, 4C, 8 weeks
Conc. (µg/g)
Other EPA Priority PAHs
Naph C1-N C2-N C3-N C4-N Phen C1-P C2-P C3-P C4-P Diben C1-D C2-D C3-D Fluo C1-F C2-F C3-F Chry C1-C C2-C C3-C
Bph Acl Ace An Fl Py BaA BbF BkF BeP BaP Pe IP DA DgP
#21, Wabamun Sterile Control with Sediment, 4C, 8 weeks
Conc. (µg/g)
Other EPA Priority PAHs
Naph C1-N C2-N C3-N C4-N Phen C1-P C2-P C3-P C4-P Diben C1-D C2-D C3-D Fluo C1-F C2-F C3-F Chry C1-C C2-C C3-C
Bph Acl Ace An Fl Py BaA BbF BkF BeP BaP Pe IP DA DgP

Figure 6 PAHs distributin

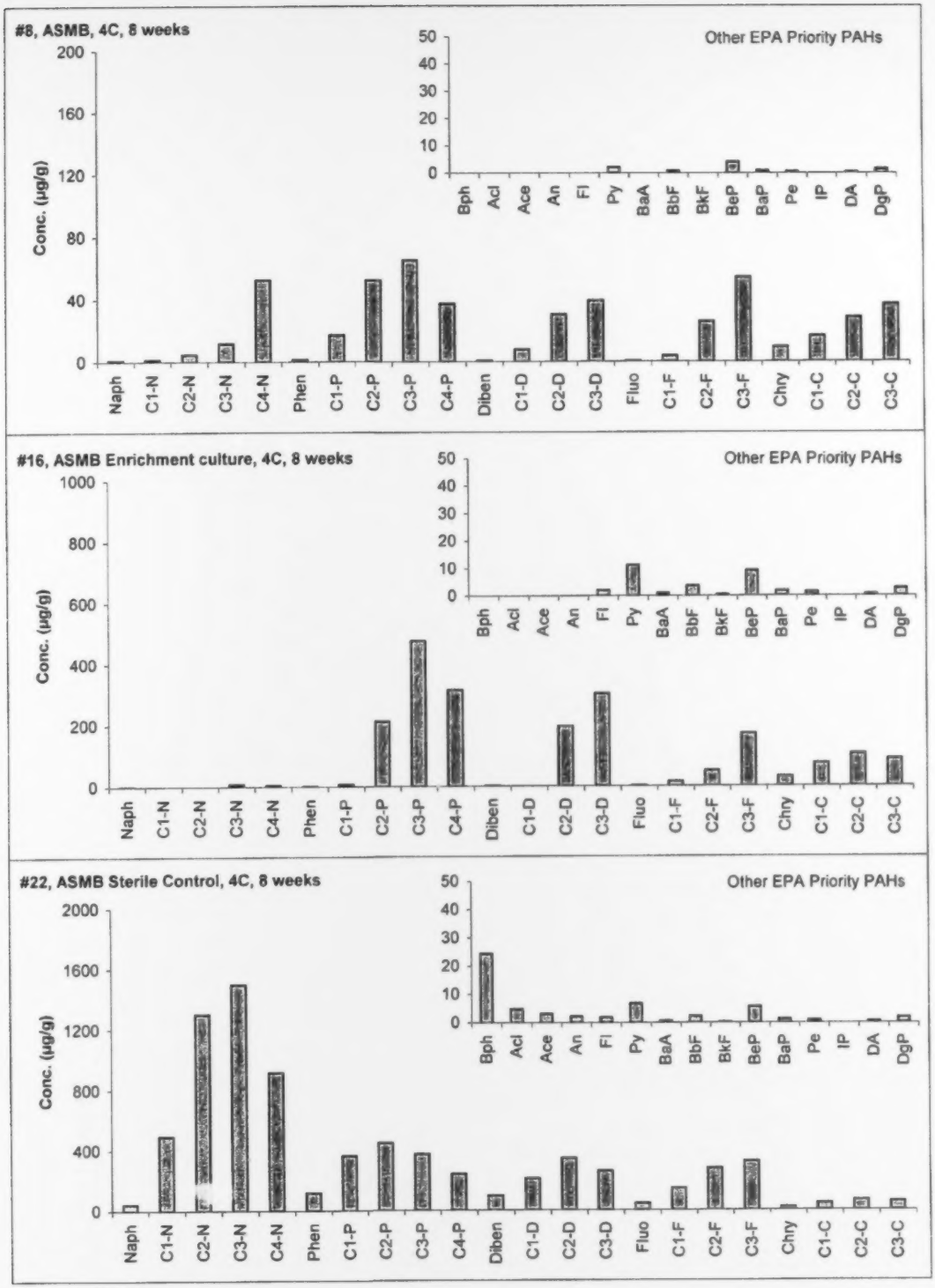
#8, ASMB, 4C, 8 weeks
Conc. (µg/g)
Other EPA Priority PAHs
Naph C1-N C2-N C3-N C4-N Phen C1-P C2-P C3-P C4-P Diben C1-D C2-D C3-D Fluo C1-F C2-F C3-F Chry C1-C C2-C C3-C
Bph Acl Ace An Fl Py BaA BbF BkF BeP BaP Pe IP DA DgP
#16, ASMB Enrichment culture, 4C, 8 weeks
Conc. (µg/g)
Other EPA Priority PAHs
Naph C1-N C2-N C3-N C4-N Phen C1-P C2-P C3-P C4-P Diben C1-D C2-D C3-D Fluo C1-F C2-F C3-F Chry C1-C C2-C C3-C
Bph Acl Ace An Fl Py BaA BbF BkF BeP BaP Pe IP DA DgP
#22, ASMB Sterile Control, 4C, 8 weeks
Conc. (µg/g)
Other EPA Priority PAHs
Naph C1-N C2-N C3-N C4-N Phen C1-P C2-P C3-P C4-P Diben C1-D C2-D C3-D Fluo C1-F C2-F C3-F Chry C1-C C2-C C3-C
Bph Acl Ace An Fl Py BaA BbF BkF BeP BaP Pe IP DA DgP

Figure 6 PAHs distributin

Table 5. Chemical composition changes of hydrocarbon groupings

Sample	Wabamun						ASMB		
	Fresh sediment, 4C, 8 weeks		Enrichment culture, 4C, 8 weeks		Sterile Control with Sediment, 4C, 8 weeks		ASMB, 4C, 8 weeks	ASMB Enrichment culture, 4C, 8 weeks	ASMB Sterile Control, 4C, 8 weeks
	n=3	RSD (%)	n=3	RSD (%)	n=2	RSD (%)	n=1	n=1	n=1
Hydrocarbons									
TPH (mg/g sample)	172	9.1	161	6.8	234	0.6	133	250	315
TSH (mg/g sample)	119	7.4	108	7.2	133	0.2	100	175	230
TAH (mg/g sample)	52.9	16.1	53.3	6.1	102	1.7	33.0	76.0	85.0
n-Alkane (mg/g sample)	1.75	77.2	0.65	125	8.41	1.5	1.33	1.08	41.8
Total 5-PAH hmologues (µg/g sample)	9692	38.3	11684	23.0	30148	7.7	494	2482	7690
Total other 15 USEPA priority PAHs (µg/g sample)	51.6	36.3	55.6	48.3	214	13.7	10.2	34.2	55.5
Hydrocarbon lost									
TPH (%)	26.5		31.2				57.8	20.6	
TSH (%)	10.5		18.8				56.5	23.9	
TAH (%)	48.1		47.7				61.2	10.6	
n-Alkane (%)	79.2		92.3				96.8	97.4	
Total 5-PAH hmologues (%)	67.9		61.2				93.6	67.7	
Total other 15 USEPA priority PAHs (%)	75.9		74.0				81.6	38.4	